An Assessment of Animal Repellents in the Management of Vehicle-Macropod Collisions in New South Wales

Submitted by
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All research procedures reported in the thesis received the approval of the relevant Ethics/Safety Committees (where required)

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Abstract

Collisions between animals and motor vehicles are frequent and often result in animal mortality. In Australia, macropods are regular victims of these collisions. This has serious implications for animal welfare and conservation as well as aesthetics and tourism. Collisions with large animals and secondary collisions caused by the presence of animals on road easements, can lead to serious personal injury and property damage. A range of mitigative measures to prevent animal-vehicle collisions exists, but no single measure can be fully effective and the efficacy of many mitigation measures remains untested. An integrated management approach, employing many mitigative techniques is required to reduce vehicle-animal collisions. Repellents have recently been identified as a potential mitigative measure for reducing vehicle-animal collisions.

The aim of this study was to identify the potential role of repellents in reducing macropod-vehicle collisions in New South Wales. This required the identification and assessment of potential repellents since research investigating repellents in an Australian context is scant. *Macropus rufogriseus banksianus* was selected as a test species for this research as a high abundance of this species exists in southeastern Australia and it is a common victim of roadkill in New South Wales.

Preliminary screening trials of four potential macropod repellents highlighted the utility of two of the substances: Plant Plus, a synthetic compound based on the chemistry of dog urine; and a formulation consisting of chicken eggs. Feeding by *M. rufogriseus banksianus* was significantly reduced when these substances were applied near feed trays. Modest results were also detected for Δ^3-isopentenyl methyl sulfide (a constituent of fox urine), while a commercial animal repellent (SCAT® Bird and Animal Repellent) was ineffective in altering feeding by *M. rufogriseus banksianus*.

A barrier trial conducted with the two most successful repellents indicated that Plant Plus was a more effective macropod repellent then the egg formulation. Plant Plus displayed qualities of an area repellent and elicited a stronger response from *M. rufogriseus banksianus* when compared to the egg formulation.

Further captive trials determined that the habituation of response to Plant Plus by *M. rufogriseus banksianus* was minimal after six weeks of constant exposure and Plant Plus retained repellent properties after exposure to ambient environmental conditions for at least ten weeks. Field trials to establish the effectiveness of Plant Plus with free ranging macropods (*M. rufogriseus banksianus* and *M. giganteus*) were unsuccessful due to methodological limitations stemming from high background variance in observed responses, equipment failure and site disturbance from outside influences.

The potential role of Plant Plus as a repellent for managing macropod-vehicle collisions was highlighted by the captive trials. However, several factors requiring further research were identified. This included assessing the repellent abilities of Plant Plus in the field and further defining the properties of Plant Plus with captive trials. The effects of Plant Plus on non-target species and an assessment of potential environmental impacts also requires attention.

Research assessing the potential role of repellents in other management contexts in Australia would be beneficial and the identification and assessment of repellents for other species should proceed. However, in the context of assessing repellents for use in the management of vehicle-macropod collisions, immediate focus should concentrate on extending the research to assess the effects of Plant Plus with other species of large macropod, and assessing if Plant Plus can reduce the numbers of macropods in road easements.

Presentations & Manuscripts in Preparation

Journals articles in preparation relevant to this thesis:

Gibson, C.P. & Wilson, S. (in prep). Habituation of *Macropus rufogriseus banksianus* to an odorous repellent. *Animal Behavior*.

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Chapter 1 Introduction

1.1 Scope

In March 2001, a discussion between representatives of the New South Wales (NSW) Roads and Traffic Authority (RTA: Bruce McNamara and Bruce Lean) and representatives of the Australian Catholic University (ACU: Dr Scott Wilson, Dr Vaughan Monamy and Craig Gibson) regarding roadkill mitigation, resulted in a mutual agreement that there was need for research into a new and innovative technique to mitigate the problem of macropod-vehicle collisions.

The research project began in July 2002 with a contribution of funds from the RTA and the ACU. Following the presentation of preliminary results to the RTA in October 2002, the RTA decided to fund the project for a further two years from July 2003.

The purpose of the project was to assess the potential of animal repellents for use in the management of vehicle-macropod collisions. Specifically, to assess if there was an effective repellent for use with macropods and if there was any potential in using it to reduce the numbers of macropods in road easements. This research is new to Australia and if repellents were found to have potential for this purpose, this research would form a basis of future work allowing the development of a mitigation strategy, including the assessment of any other potential effects that repellents could have on the environment.

All research conducted as part of this thesis was designed according to the principles outlined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH&MRC, 2004) and relevant legislation that relates to the use of animals for scientific purposes in NSW. No animal was subjected to pain or discomfort. Copies of appropriate ethics approvals and National Parks and Wildlife Service Permits are located in Appendix A.

1.2 Background

1.2.1 Roadkill

Roads have many ecological effects (Trombulak & Frissell, 2000) and "Road Ecology" is now the subject of several texts (Forman *et al.*, 2002; Spellerberg, 2002). The literature relating to road ecology is extensive, with the Wildlands CPR bibliographic database (accessible and searchable at http://www.wildlandscpr.org/bibliographic-database-search) containing over 12 000 citations specifically relating to road ecology (August 2008). Roadkill (animal mortality due to animal-vehicle collisions) is a conspicuous and often reported phenomenon and surveys of roadkill have been published since the early to mid twentieth century (Stoner, 1925; Dickerson, 1939; Shadle, 1940; Huey, 1941; Haugen, 1944; Hawbecker, 1944; De Vos, 1949). Surveys of roadkill are numerous and have been published for many locations and species, and the annual amount of wildlife roadkill for each nation is often extrapolated from these surveys (Forman & Alexander, 1998; Erritzoe *et al.*, 2003). It has been widely reported that the best estimate of the roadkill rate in the United States of America (USA) is one million vertebrates per day (Lalo, 1987) with a similar number being reported for continental Europe (Forman & Alexander, 1998). In Australia, a reliable figure is not yet available although it has been estimated that 5.4 million frogs and reptiles are killed on roads annually (Ehmann & Cogger, 1985).

Vehicle collisions with wildlife also impact humans. Putman (1997) reviewed the significant incidence of human deaths due to vehicle collisions with wildlife in Sweden, Germany, Norway and the USA and the costs associated with property damage and loss (e.g. insurance claims, medical expenses). In Australia, Coulson (1985) reported three human deaths occurring in Victoria between 1977 and 1983 resulting from vehicle collisions with kangaroos or wombats. Occasionally, human deaths resulting from vehicle-macropod collisions are reported in newspapers including a recent fatal accident in Western Australia (AAP, 2006). Serious injuries to drivers and passengers in Australia have also been reported more recently (NRMA, 2003a; Magnus *et al.*, 2004). The average cost of damage to vehicles from each collision with an animal in Australia is more than $3000, totalling $21 million nationally in 2002 (NRMA, 2003b). In addition to the property damage and injury costs associated with roadkill, there are also effects on tourism which can be costly for wildlife based tourism operators (Magnus *et al.*, 2004).

Traffic (speed and volume), road (structure and surface type), landscape (vegetation and topography), season (solar and lunar) and weather have all been identified as factors influencing the occurrence of roadkill (Hodson, 1962; McCaffery, 1973; Puglisi *et al.*, 1974; Coulson, 1982; Adams, 1984; Bashore *et al.*, 1985; Davies *et al.*, 1987; Osawa, 1989; Forman & Alexander, 1998; Finder *et al.*, 1999; Clevenger *et al.*, 2003; Nielsen *et al.*, 2003). Species behaviour and ecology are also major factors influencing the occurrence of roadkill (Hodson, 1962; Puglisi *et al.*, 1974; Tabor, 1974; Jefferies, 1975; Fremlin, 1985; Carr & Fahrig, 2001; Dale, 2001). There are also many other locally (e.g. topographic) or species specific (e.g. biological, behavioural) factors that can influence roadkill occurrence (Bennett, 1991; Forman *et al.*, 2002). An understanding of the reasons why animals move onto roads is necessary to predict and prevent the occurrence of roadkill. However, due to the involvement of many factors, roadkill is highly variable both spatially and temporally and surveys are locally, species and temporally specific (Case, 1978; Bennett, 1991; Jaeger *et al.*, 2005). However, roadkill surveys are useful in identifying areas of specific importance or where further management is required (Jaeger *et al.*, 2005; Seiler, 2005).

1.2.1.1 Roadkill in Australia

Growing scientific interest in road ecology and roadkill in Australia is evident from a growing number of symposia and publications. Numerous roadkill surveys and studies have been conducted (Appendix B) and an adequate review can be found in Donaldson & Bennett (2004). However, several studies have been published since the completion of the review (Lee *et al.*, 2004; Taylor & Goldingay, 2004). The wide variety of work conducted on roadkill in Australia is evident from Appendix B.

In New South Wales, roadkill surveys and studies have been conducted with various objectives (Vestjens, 1973; Disney & Fullagar, 1978; Thomas, 1988; Lepschi, 1992; Cooper, 1998; Lee *et al.*, 2004; Morrissey, 2004; Taylor & Goldingay, 2004). Most of this work has focused on rural and regional areas. However, Morrissey (2004) surveyed roadkill in the Royal National Park (within the Greater Sydney Region) for five months in 2003.

Vertebrate roadkill surveys in Australia have found roadkill rates between 0.26 roadkill/kilometre/day (Taylor & Goldingay, 2004) and 0.035 roadkill/kilometre/day (Morrissey, 2004). Cooper (1998) calculated a roadkill rate of 0.05 roadkill/kilometre/day for some roads in New South Wales and Ramp (2004) extrapolated this equation to give a rate of 7000 animals killed on roads per day in New South Wales. The ability for an accurate extrapolation from the original data (Cooper, 1998) is questionable as sampling was very limited and selective.

There are more than 800 000 kilometres of road in Australia, with over 12 million registered vehicles travelling more then 173 billion kilometres annually (Austroads, 2000). New South Wales contains more than 180 000 kilometres of road with 3.8 million registered vehicles travelling over 55 billion kilometres/year. By extrapolating the data of Taylor & Goldingay (2004) and Morrissey (2004) with the physical features of the roads in Australia (Austroads, 2000), estimates of annual roadkill in Australia are between 10 million and 76 million birds and mammals, with between 2.3 million and 17 million in New South Wales. A further extrapolation of data from Cooper (1998), that includes reptiles but not amphibians, is at the lower bounds of these figures (2.5 million roadkill/year). Additionally, Ehmann and Cogger

(1985) estimated that at least 5.4 million frogs and reptiles are killed on Australian roads annually. It is important to note that there are no standard survey methods for roadkill and each study has inherent biases (number of observers, mode of traversing route (on foot, in vehicle – speed variations), time of day surveyed, extent of roads surveyed etc). As such, there are many sources of error in these extrapolations.

Roadkill in Australia and New South Wales is clearly responsible for a large amount of wildlife mortality and has serious implications for animal welfare and conservation, personal injury, property loss, tourist perceptions and aesthetics (Vestjens, 1973; Committee, 1997; Lintermans & Cunningham, 1997; Patience, 2000; NRMA, 2003b; Magnus *et al.*, 2004). Macropods in particular can cause serious injury and property damage as they are frequently reported in vehicle-wildlife collisions and are relatively large (Coulson 1982; 1985). Therefore mitigation of vehicle-macropod collisions is of importance.

Some factors associated with the occurrence of macropod roadkill have been identified and include landscape, seasonal, climatic and generalised behaviour; however, some factors are spatially and temporally specific (Coulson 1982; Lee *et al.*, 2004; Osawa, 1989). Disproportionately large numbers of juvenile male macropods are found killed on roads and this has been attributed to the increased tendency of this age/gender cohort to disperse (Coulson, 1997). The abundance of food and water resources within road easements, particularly in drought, has also been reported as a contributing factor to vehicle/macropod collisions (Coulson 1989; 1997).

1.2.1.2 Roadkill Mitigation

Advances in roadkill mitigation have often stemmed from surveys and trials specifically studying deer-vehicle collisions in the northern hemisphere. The mitigation of deer-vehicle collisions in particular has received much attention due to the high collision rate (Knapp *et al.*, 2003) and the damage and danger caused by such collisions (Putman, 1997). There are several broad descriptive reviews of mitigation techniques specifically for use by road transport planners to reduce deer roadkill (Danielson & Hubbard, 1998; Schwabe *et al.*, 2003; Hedlund *et al.*, 2004; Knapp *et al.*, 2004). However, scientific reviews of the effectiveness of mitigative measures are limited (Romin & Bissonette, 1996; see Chapter 6 of Forman *et al.*, 2002).

Wildlife fencing (Clevenger *et al.*, 2001), over and under passes (Mansergh & Scotts, 1989; Yanes *et al.*, 1995; Bruinderink & Hazebroek, 1996; Norman *et al.*, 1998; Clevenger & Waltho, 2000), hazing (periodically spooking animals that encroach on easement: Romin & Bissonette, 1996), habitat alteration (Rea, 2003), mirrors and reflectors (Schafer & Penland, 1985; Lintermans, 1997; Nolan & Johnson, 2001), warning signs (Pojar *et al.*, 1975; Coulson, 1982), ultrasonic whistles (Romin & Dalton, 1992; Bender, 2003), public education (Bruinderink & Hazebroek, 1996; Putman, 1997), highway lighting (Reed & Woodard, 1981) and reduced speed limits (Jones, 2000) are all mitigation techniques that have been used in attempts to reduce roadkill. A range of other techniques (in-vehicle technologies and herd reduction) have also been proposed (Knapp *et al.*, 2004).

The evaluation of mitigation methods is often post hoc or scant (Knapp *et al.*, 2003; Hedlund *et al.*, 2004). However, both speed reduction and exclusionary fencing (with under or overpasses to mitigate habitat fragmentation) have been appropriately evaluated and found to be effective in reducing roadkill of some species in several locations (Clevenger & Waltho, 2000; Clevenger *et al.*, 2001; Taylor & Goldingay, 2003). Romin & Bissonette (1996) surveyed state-based natural resource agencies in the United States of America (USA), collecting data on the usage, perceptions and research of deer-vehicle collisions and mitigative strategies. Agencies from 43 states responded to the survey and 11 different mitigation strategies were found to be in use. Romin & Bissonette (1996) noted that appropriate evaluation of the effectiveness of most strategies was lacking, and for the few strategies that had been appropriately researched and tested, the results of the evaluations were largely ignored. It was concluded that further research was required for most mitigative strategies and communication and dissemination of results between agencies on the effectiveness of measures needed improvement.

Recently, repellents have been suggested as a roadkill mitigation strategy. The use of repellents in roadkill mitigation will be further discussed in Section 1.2.3.

1.2.1.3 Roadkill Mitigation in Australia

In Australia, roadkill mitigation research and application, has started to advance rapidly, particularly in Tasmania where a recent study and review of roadkill mitigation measures has been completed (Magnus *et al.*, 2004; Magnus, 2006). It is speculated that Tasmania has the highest state-wide roadkill incidence rate in Australia and there is much conjecture in Tasmania about the negative impacts of roadkill on tourism and wildlife tourism operators (Magnus *et al.*, 2004). Wildlife signage, escape routes, drain (ditch) management, platypus crossings and underpasses were mitigation measures identified as having the most potential for reducing roadkill. The use of odour repellents was also suggested pending further research (Magnus *et al.*, 2004).

A review of the management of kangaroos along roadsides in the Australian Capital Territory (ACT) concluded that no current mitigation technique was fully effective in reducing vehicle-kangaroo collisions (Committee, 1997). The cost-effectiveness of mitigation measures was also highlighted as excessive. The report recommended a program to focus on driver and community behaviour, but also encouraged further research for deterrent devices, including repellents (Committee, 1997).

Jones (2000) reported the recovery of a local population of *Dasyurus viverrinus* (eastern quoll) following the implementation of slow points, wildlife signage, swareflex reflectors and drainage line escape routes on an access road to Cradle Mountain, Tasmania. This was following a population crash of both *D. viverrinus* and *Sarcophilus harrisii* (Tasmanian devil) in the area after a major road upgrade that led to increased traffic densities and speeds. There was also some evidence of *S. harrisii* recovering after the installation of mitigation measures (Jones, 2000).

Overpasses and underpasses with fauna exclusion fencing are a common roadkill mitigation strategy employed by the NSW RTA on newly constructed or upgraded major highways (Roads and Traffic Authority, 2005). Mansergh & Scotts (1989) reported on the success of a tunnel under a road on Mt Higginbotham, Victoria. The tunnel reconnected fragmented habitat for *Burramys parvus* (mountain pygmy possum) and reduced a critical population decline. The tunnel design incorporated furnishings (a boulder field) and the success of the tunnel following the addition of furnishings has increased the understanding and success rate of wildlife underpasses.

An earlier study on mammal use of culverts under a railway line in NSW reported the utilisation of established tunnels by small mammals (Hunt *et al.*, 1987). However, all new tunnels surveyed by Hunt *et al.* (1987) were used predominantly by feral predators and it was predicted that small mammal use of tunnels and culverts was reliant on the regeneration of vegetation around tunnel/culvert entrances.

Different types of tunnels and culverts running under the F3 freeway, north of Sydney, NSW were found to facilitate movement of a range of animals (Norman *et al.*, 1998). Underpasses ranging from 1.5m to 10m in diameter were studied and it was reported that the greatest range of native species used the largest size underpass, however, more movements were recorded through the smaller underpasses. While the study only observed animal use of a small number of tunnels, it was concluded that underpasses of various sizes and designs played a significant role in facilitating the safe movement of animals across a road easement (Norman *et al.*, 1998). Taylor & Goldingay (2003) confirmed these findings in north-eastern NSW reporting frequent use of underpasses by a variety of native species. Fauna exclusion fencing was also found to be successful in directing most animals away from the roadway and in many cases into underpasses (Taylor & Goldingay, 2003). Seventeen vertebrate species were detected using the underpasses including 12 mammalian species, however, many frogs were found dead on the roadway.

Overpasses increased the success rate of road crossings for several mammal species in the wet tropics of Queensland (Goosem & Turton, 2000). This design of overpasses is now popular amongst road management authorities and overpasses for arboreal mammals are now used for roadkill mitigation in NSW (Roads and Traffic Authority, 2005).

The efficacy of the 'Shuroo' (Shuroo Australia Pty Ltd), an ultrasonic whistle designed for attachment to vehicles with the purpose to scare macropods away from roads, was assessed by Bender (2001; 2003). The device was tested acoustically, and its effects on macropods were tested by captive and field trials with two species. An additional trial involving the attachment of devices to 58 vehicles was also conducted. Bender (2001; 2003) concluded that the Shuroo emitted both audible and ultrasonic sounds, however, they were not detectable 400m from the source. It was also concluded that the Shuroo signal did not alter the behaviour of *Macropus giganteus* or *M. rufus*. There was no evidence that frequencies emitted were detectable by either *M. giganteus* or *M. rufus* and the Shuroo did not reduce animal densities in field trials or make a difference to collision rates when attached to vehicles (Bender, 2001; 2003).

Specialised roadside reflectors were used in Queensland to reduce the number of road deaths of *Petrogale persephone* (Proserpine rock-wallaby) and the reflectors appeared to be effective (Nolan & Johnson, 2001). Reflectors were also installed along a stretch of road in Tasmania and assessed by Jones (2000). The reflectors were installed at the same time as several other mitigation measures, and overall, the mitigation measures were successful in allowing the recovery of two species of dasyurid. Unfortunately, the trial was not designed to test the effectiveness of the reflectors, but to assess the impacts of the road on the dasyurid species present, and a true assessment of the reflectors efficacy is lacking (Jones, 2000).

Lintermans (1997) reported that a properly designed road-based trial was yet to be conducted in Australia for reflectors and such a study would be cost inhibitive and time consuming. The author suggested a trial to assess the response of macropods to reflectors to determine if the wavelength of light reflected is detectable by macropods. Recent research by David Croft and Daniel Ramp at the University of New South Wales (UNSW) addressed some of these issues and research has indicated that the response of macropods varies between species (Ramp & Croft, 2002).

In southern Queensland, differential speed signs (similar to those used around schools in NSW) were used to assess if a reduction in speed limit during the breeding season of *Phascolarctos cinereus* (koala) would result in a reduced number of *P. cinereus* roadkill (Dique *et al.*, 2003). The change in speed limit did not alter the average speed of vehicles despite routine enforcement of speed zones by Queensland Police. Consequently, an assessment of the effects of reduced speed on *P. cinereus* roadkill was not possible (Dique *et al.*, 2003). Similarly, during an assessment of macropod roadkill in central Victoria, kangaroo collision signs were erected and Coulson (1982) reported that they were not effective in reducing the rate of roadkill. Numerous causal factors were highlighted relating to *P. cinereus* roadkill and it was suggested that vehicle speed may not have a large role (Dique *et al.*, 2003). However, further study of the *P. cinereus* populations around the study zone, the characteristics of *P. cinereus* roadkill hotspots, and the impacts of traffic on *P. cinereus* were suggested (Dique *et al.*, 2003). Coulson (1982; 1985; 1989; 1997) also indicated many causal factors for collisions with macropods may exist and could include season, lunar cycle, landscape and vegetation.

1.2.2 Repellents

Chemical and biological repellents have been used with varying success for forestry and agriculture purposes for several decades (Dietz & Tigner, 1968; Muller-Schwarze, 1972; Stoddart, 1976). The main use of repellents has been to reduce damage caused by herbivores to crops, nursery plants, regenerating forests and old growth forests. However, they have also been used successfully to protect underground cables from gopher damage (Shumake *et al.*, 1999). Mammal repellents are generally categorised by the mode of action (fear, conditioned aversion, pain, or taste) and the mode of application (systemic, topical or area-based: Beauchamp, 1995; Wagner & Nolte, 2001 Nolte, 2003).

The main constituents of odoriferous animal repellents vary (Muller-Schwarze, 1990; Bean *et al.*, 1995). Some of the most effective animal repellents have been produced from putrescent egg solids (Bullard *et al.*, 1978), however success has also been achieved using predator odours (for a review see Apfelbach *et al.*, 2005), plant-based (Crocker, 1990; Watkins *et al.*, 1994; Avery *et al.*, 1996; Gurney *et al.*, 1996) and synthetic sulfur-based odours (Bullard *et al.*, 1978; Lindgren *et al.*, 1995; Burwash *et al.*, 1998b).

Repellents can remain effective for between 3 and 12 months (dependent on the repellent) if sprayed directly as a solution. However, if repellents are microencapsulated, they can be made into pastes or used to coat or impregnate textiles, paper or metal strips (Boh *et al.*, 1999). Some repellents can be introduced to plastics during the co-polymerisation stage in manufacture. Repellents have been found to be more effective and last longer (>12 months) if applied microencapsulated (Boh *et al.*, 1999).

There are a number of odoriferous repellents commercially available in North America, Europe, New Zealand and Australia. These repellents have been found to be very effective on a wide variety of animals (particularly herbivores) including *Trichosurus vulpecula* (common brushtail possum). Appendix C contains a partial review of work that has involved the investigation of repellents with mammalian species. A wide variation in the range of species and repellents, as well as the response elicited can be seen. Mammals were repelled in approximately 80% of the 300 experimental situations (Appendix C).

Olfaction is a major contributor to animal awareness (Sommerville & Broom, 1998) and most odour repellents rely on inducing a fear or defensive response in the target species. The underlying mechanisms for the responses to predator odours vary from species to species but can be innate or learned defensive responses. Alternatively, predator odours can affect palatability of food, alter reproductive capacity and provide a range of other cues, which may affect response (see Takahashi *et al.*, 2005 and Apfelbach *et al.*, 2005 for review). Some odour repellents rely instead on irritation of the target species (Andelt *et al.*, 1994). The use of predator odours as repellents has recently been reviewed (Apfelbach *et al.*, 2005) and the use of synthetic semiochemicals was reviewed by Lindgren (1995). Lindgren *et al.* (1995) highlighted the success of several captive based and small-scale field trials of various repellents with several species of mammals, while also elucidating the lack of consistent results in large scale experiments with commercially important species (e.g. *Odocoileus hemionus columbians*: black tailed deer). The observed responses to some odours selected were found to be innate, but in some cases genetically controlled, while other responses seemed to be the result of pre-conditioning (Lindgren *et al.*, 1995). Some research has investigated the influence of habitat and environment in relation to the effectiveness of repellents (e.g. effect of available resources: Andelt *et al.*, 1992), however, these important influences received little attention in most studies (Lindgren *et al.*, 1995). Further recommended research included investigating the efficacy of repellents under different weather conditions, with varying concentrations of repellent and on a broader range of herbivores. The length of effect was also deemed to be a priority research topic (Lindgren *et al.*, 1995).

Apfelbach *et al.* (2005) indicated that the repellent effects of some predator odours could be of practical use in the management of pest mammals: however, there were several examples where odours failed to elicit the desired results (see Appendix C for examples). Apfelbach *et al.* (2005) highlighted three common behavioural responses to predator odours that are useful when considering predator odours as repellents. These responses are:

1) Inhibition of activity;
2) Suppression of feeding, grooming and foraging (and other non-defensive behaviours); and
3) Avoidance response.

Apfelbach *et al.* (2005) deduced that predator odours may be successful as repellents as they could prevent target species from entering forestry and agricultural areas and could also reduce foraging or feeding in such areas. However, the lack of response to odours in several studies was problematic. Some explanations for these negative results have been suggested and include: the selection of inappropriate odours; incorrect presentation or context; inappropriate odour concentrations; and rapid habituation by subjects (see Apfelbach *et al.*, 2005 and Takahashi *et al.*, 2005 for reviews).

Effective repellents used to deter feeding by *T. vulpecula* in New Zealand have included synthetic odorous chemicals, derived and/or manufactured to mimic the odours of predatory mammalian species including the red fox (*Vulpes vulpes*), and commercial egg formulations. The former, a commercial formulation labelled Pine Plus, was found to be a very effective repellent to possums and rabbits (Morgan & Woolhouse, 1995; Woolhouse & Morgan, 1995; Morgan & Woolhouse, 1998). Cooney (1998) also investigated the efficacy of several repellents with *T. vulpecula* and found several effective (White King®, Keep Off®, Camphor, Naphthalene, Scat®) and several ineffective agents (Tabasco sauce®, Hot English mustard, Indonesian fish sauce, Bitrex, Garlic spray, D-Ter®, Stay Off®, Blood and Bone, Quassia chips). The use of odours as lures to assist in the capture of *T. vulpecula* was investigated by Todd *et al.* (1998), however no effective substances were reported.

Investigation into repellents for use with other Australian mammals has been limited, however, promising research has been conducted with *Macropus parma* (parma wallaby) and *Thylogale thetis* (red-necked pademelon: Ramp *et al.*, 2005), *Wallabia bicolor* (swamp wallaby: Montague *et al.*, 1990; Montague, 1994) and *Pteropus poliocephalus* (grey headed flying fox: Van Der Ree & Nelson, 2002). Recently, some promising research with *M. fuliginosus* (western grey kangaroo) has also been conducted (Parsons *et al.*, in press). There has also been preliminary investigations into the use of repellents with feral animals in Australia (Murray *et al.*, 2006).

A synthetic predator odour (Plant Plus: Roh Koe and Associates Pty Ltd) was investigated by Ramp *et al.* (2005) with *M. parma* and *T. thetis*. Pine Plus, investigated by Woolhouse & Morgan (1995) was an earlier formulation (preceding Plant Plus) and both products are based on the chemistry of dog urine (Thomas Montague, Roh Koe and Associates Pty Ltd, pers. comm.). Although canine odour was a novel odour for *M. parma* and *T. thetis* (subjects had no reported previous contact with canines), a defensive/anti-predator response was reported for each species. However, the response of *T. thetis* was different than the response of *M. parma*. *Macropus parma* was repelled by Plant Plus and spent significantly less time in areas where Plant Plus was present. Whereas the response of *T. thetis* was to investigate the odour (not significant but similar to a response to predator odour reported by Blumstein *et al.*, 2002 for *T. thetis*). Ramp *et al.* (2005) proposed that both responses were anti-predator and differed due to differing species ecology.

Montague *et al.* (1990) screened 18 potential repellent formulations with *W. bicolor*. Only two repellents (dog urine and chilli) significantly reduced browsing damage to *Eucalyptus* *regnans* seedlings in captive trials. Field trials conducted with dog urine revealed that browsing on *E. regnans* by *W. bicolor* was reduced by up to 50% following six weeks (Montague *et al.*, 1990). Similarly, Parsons *et al.*, (in press) reported the effectiveness of canine urine in repelling *M. fuliginosus*. The active ingredient of dog urine responsible for the repellent properties was not identified by either study and it was suggested that a fear response was elicited in the target species of both studies (*W. bicolor* and *M. fuliginosus*).

The relative palatability of *Eucalyptus spp.* seedlings for *W. bicolor* was investigated by Montague (1994). The effectiveness of two repellents in effecting seedling palatability was also investigated. Selenium was found to reduce seedling palatability for *W. bicolor*, but also stunted seedling growth and resulted in high seedling mortality rates. Treatment of seedlings with Bitrex (denatonium benzoate) also stunted seedling growth with no reduction in browsing by *W. bicolor* noted (Montague, 1994).

A capsaicin based commercial repellent (Envirospray Ultrawax Flying-fox repellent: Envirocare Technologies Australia) was evaluated with *P. poliocephalus* in the Royal Botanic Gardens, Melbourne (Van Der Ree & Nelson, 2002). *Pteropus poliocephalus* roost in large numbers and cause significant damage to some vegetation in the roost camp. A weak response by *P. poliocephalus* to the repellent was detected in a preliminary trial. However, the effect was slight and a second trial investigating animal abundance did not detect any effect. At the concentrations trialled, Envirospray was not effective enough to be used as a management tool (Van Der Ree & Nelson, 2002).

1.2.3 Repellents in Roadkill Mitigation

Recently repellents have been suggested as a roadkill mitigation strategy but little is known of their effectiveness (Hedlund *et al.*, 2004; Knapp *et al.*, 2004; Magnus, 2006). Brown *et al.* (2000) investigated the repellence of three compounds to *Rangifer tarandus* (caribou) with the intention that they could be used in road de-icing salt and sand mixtures as a way of reducing vehicle collisions in Canada. Lithium chloride (LiCl: a gastrointestinal toxicant) was found effective in reducing the amount of food, the number of feeding bouts and the total time spent feeding for *R. tarandus*. Brown *et al.* (2000) suggested that field trials should proceed to assess if salt-sand mixtures containing LiCl can reduce the amount of time *R. tarandus* spent on roads licking salt and also the number of animal-vehicle collisions.

The Insurance Corporation of British Columbia commissioned an investigation into the use of area-based repellents to reduce wildlife-vehicle collisions (Kinley & Newhouse, 2004). The authors tested three area repellents (Deer Away®, C*anis latrans* (coyote) urine and *C. latrans* anal gland secretion) in a field trial and recorded the responses of *Odocoileus hemionus* (mule deer), *O. virginianus ochrourus* (white-tailed deer) and *Cervus elephus nelsoni* (elk). No significant effects of the repellents were detected, however the statistical power of the study was low, and there was limited evidence that at least one of the repellents had some ability to repel *O. virginianus ochrourus* (Kinley & Newhouse, 2004). Further research is required to assess the potential of the repellents in reducing vehicle accidents.

Putman (1997) reported two German studies that investigated the use of repellents to create scent-fences to mitigate roadkill. Kerzel & Kirchberger (1993 in Putman, 1997) reported that the number of roe deer killed on a section of road decreased following treatment with a scent-fence. It was also reported that 60% of animals approaching the scent-fence withdrew and only crossed the road at an untreated section. Kerzel & Kirchberger (1993 in Putman, 1997) also reported that of the remaining 40% of animals that crossed the road, half crossed rapidly without delay, reducing the time spent on road. The objectivity of the trials has been questioned as Kerzel & Kirchberger (1993 in Putman, 1997) were the manufacturers of the product used as the scent-fence, and Lutz (1994 in Putman, 1997) found that the fences were not as effective as the manufacturers claimed. However, recent review articles have suggested that repellents could be effective in mitigating roadkill and warrant further research (Knapp *et al.*, 2004; Magnus *et al.*, 2004).

There are several ways in which repellents could be used to reduce vehicle-macropod collisions in New South Wales: An appropriate repellent may reduce densities or increase vigilance behaviour of macropods in road easements that may decrease the likelihood of macropod-vehicle collisions; A feeding deterrent could reduce the palatability of resources in road easements, decreasing the visitation rate of macropods to roadsides; or a scent fence may be able to restrict the movement of animals through road easements. There is limited evidence of the effectiveness of repellents for mitigating roadkill; however, this area of research has been highlighted as important and forms the basis of this thesis.

1.3 Macropod Test Species – *Macropus rufogriseus banksianus*

Two sub species of *Macropus rufogriseus* have been described. *Macropus rufogriseus rufogriseus* (Bennett's Wallaby) is found only in Tasmania and on the Islands of Bass Strait (Calaby, 1983). *Macropus rufogriseus banksianus* (red-necked wallaby) is an abundant macropod in southeastern Australia and a common roadkill victim in New South Wales (Greg Clancy, pers. comm.). The two subspecies have differing breeding patterns: *M. rufogriseus rufogriseus* has a well defined breeding season with births occurring from January to July, whereas *M. rufogriseus banksianus* breed all year round with a slight increase in the birth rate in summer (Calaby, 1983). In captivity, these distinct breeding patterns remain.

Macropus rufogriseus banksianus is a grazer common to eucalypt forests (with a moderate to dense shrub stratum) and tall coastal heath communities (Calaby, 1983). Essentially solitary during the day when most time is spent resting in dense shrubs, large groups may sometimes form in prime grazing areas after dark. Individuals emerge from daytime shelter in the late afternoon, but remain near shelter until after dark. However, *M. rufogriseus banksianus* may emerge and aggregate earlier on dull, wet or cooler days (Calaby, 1983).

Macropus rufogriseus banksianus is sexually dimorphic with male adult weight (15-23.7 kg) being greater than the female adult weight (12-15.5 kg: Calaby, 1983). Home ranges tend to be small and cover areas where feeding habitat (open grassy areas) and shelter (dense vegetation) are closely situated (Johnson, 1987). In a population studied at Wallaby Creek in northern NSW, the small home ranges (15.2 ha) were stable and often located near creeks. Dispersal of two-year old males were the exception to the stability of the home ranges (Johnson, 1987). Males at this study site also tended to have larger home ranges than females.

As *M. rufogriseus banksianus* are mostly solitary, groups that form in feeding areas tend to be unstable and smaller than those formed by other macropod species (Johnson, 1989a). Group sizes were noted to vary seasonally and were least stable in winter. There is contradicting evidence regarding the benefits that individuals may obtain by forming feeding groups (Johnson, 1989a, Coulson, 1999). However, the benefits obtained by other macropod species by grouping do not seem to benefit *M. rufogriseus banksianus* to the same degree.

Macropus is the largest genus (number of species) of Diprotodonta (Strahan, 1983). While *M. rufogriseus banksianus* is one of the few species of *Macropus* that is mostly solitary, the biology, ecology and behaviour of *M. rufogriseus banksianus* is very similar to most other species of *Macropus* (Calaby, 1983; Johnson, 1989b; Jarman, 1991).

Macropus rufogriseus banksianus was selected as a test species for this study due to its frequent involvement in vehicle collisions, the abundance of *M. rufogriseus banksianus* in NSW, the availability of large numbers for captive study at the UNSW Cowan Field Station, the extensive research conducted on the biology and ecology of the species (e.g. Kaufmann, 1974; Johnson, 1987; Johnson & Jarman, 1987; Johnson *et al.*, 1987; Southwell, 1987; Southwell & Jarman, 1987; Higginbottom, 1989; Johnson, 1989a; 1989b; Lunney & O'Connell, 1989; Coulson, 1999; McArthur *et al.*, 2000), and the similarities in biology and ecology of *M. rufogriseus banksianus* with other species of large macropods.

Species: *Macropus rufogriseus banksianus*

Family: Macropodidae

Super family: Macropodoidea

Order: Diprotodonta

1.4 Objectives of Research

Due to the high rates of vehicle-animal collisions in Australia and the impacts on animal welfare and the community (refer to Section 1.2.1.1), further research on mitigative measures to reduce roadkill in Australia is necessary. Macropods can cause serious injury and property damage when involved in vehicle-wildlife collisions, therefore mitigation of vehicle-macropod collisions is of particular importance. Several roadkill mitigation strategies are employed in Australia (refer to Section 1.2.1.3), including some specifically targeted at macropods (e.g. Nolan & Johnson, 2001). However, the effectiveness of these techniques have not been fully evaluated and no single mitigation technique is likely to be fully effective (Lintermans & Cunningham, 1997). A new strategy to reduce macropod-vehicle collisions is required.

The potential of animal repellents in mitigating wildlife-vehicle collisions in Australia has been recognised (Magnus *et al.*, 2004; Ramp *et al.*, 2005) and several trials to assess the role of repellents in reducing roadkill in other countries have been attempted (Kerzel & Kirchberger, 1993 in Putman, 1997; Lutz, 1994 in Putman, 1997; Brown *et al.*, 2000; Kinley & Newhouse, 2004). Animal repellents have been successfully used with mammals in several management situations, however, work with Australian mammals has been limited to only a few species. Further work identifying potential repellents for Australian mammals is required as animal repellents could have a significant role in natural resource management in Australia. The use of repellents as a roadkill mitigation technique could provide a safe, inexpensive alternative or supplement to the current engineered solutions (detailed in Section 1.2.1.2).

The objective of this research was to determine if animal repellents have potential for use in the management of vehicle-macropod collisions in NSW. This involved:

- Assessing the effectiveness of selected repellents for use with macropods; and
- Assessing the ability of repellents to reduce the number of macropods in road easements.

This research has formed a basis for future research that will develop and test repellents as a roadkill mitigation strategy.

Chapter 2.

Pilot screening trials with *Macropus rufogriseus banksianus*

Chapter 2 Pilot Screening Trials with *Macropus rufogriseus banksianus*

2.1 Introduction

2.1.1 Background

The use of odours in the management of mammal populations has a long history, particularly the use of attractants to increase trap efficiency (see Muller-Schwarze, 1990). For animal husbandry, especially with domestic stock and exotic species in zoos, odours are used to manipulate feeding behaviour and reproduction (Muller-Schwarze, 1990). More recently, repellents have been considered for wildlife damage control (refer to section 1.2.2 and Muller-Schwarze, 1990; Lindgren *et al.*, 1995; Apfelbach *et al.*, 2005). As the role of odours in food selection and feeding behaviours has been extensively studied (e.g. Dietz & Tigner, 1968; Muller-Schwarze, 1972; Bullard *et al.*, 1978; Stoddart, 1982; Abbott *et al.*, 1990; Pfister *et al.*, 1990; Arnould & Signoret, 1993; Arnould *et al.*, 1998; Tien *et al.*, 1999), many preliminary studies of potential repellents focus on feeding rates (see Appendix C and Lindgren *et al.*, 1995; Apfelbach *et al.*, 2005).

The main constituents of odoriferous animal repellents vary (Muller-Schwarze, 1990; Bean *et al.*, 1995). Some of the most effective animal repellents have been produced from putrescent egg solids (Bullard *et al.*, 1978): however, success has also been achieved using predator odours (for a review see Apfelbach *et al.*, 2005), plant-based (Crocker, 1990; Watkins *et al.*, 1994; Avery *et al.*, 1996; Gurney *et al.*, 1996) and synthetic sulfur-based odours (Bullard *et al.*, 1978; Lindgren *et al.*, 1995; Burwash *et al.*, 1998). Unfortunately, the chemical and biological complexities of repellents and semiochemicals (and the complexities of the responses they elicit) often leads to the selection of inappropriate stimuli and/or unexpected results (Albone, 1990; Apfelbach *et al.*, 2005). For these reasons, it is common to screen a number of potential substances for preliminary responses, before comprehensive studies are attempted (e.g. Conover, 1984; Sullivan & Crump, 1986; Montague *et al.*, 1990; Swihart, 1990; Andelt *et al.*, 1991; Arnould & Signoret, 1993).

Mammals can respond to a large range of novel stimuli (Albone, 1990). The biological significance of stimuli can be obstructed in experimental situations as animals often habituate, display a large variation in response (between animals and over time) and learn to respond to the test situation. To overcome these problems, large numbers of subjects are required and the test environment and the presentation of stimuli should closely resemble the natural context (Muller-Schwarze *et al.*, 1985; Albone, 1990). Unfortunately, it is impractical to use many subjects and/or a natural environment when working with some species of large mammal (Albone, 1990).

Choice-based feeding trials are good for screening a range of potential repellents and are often utilised to assess feeding preferences and the aversion created by repellents (e.g. Bullard *et al.*, 1978; Harris *et al.*, 1983; Avery *et al.*, 1992; Boag & Mlotkiewicz, 1994; Nolte *et al.*, 1994b; Nolte *et al.*, 1995; Belant *et al.*, 1997; Nolte & Barnett, 2000; Avery *et al.*, 2001; Moran, 2001). These trials have been utilised for many species, particularly large herbivores where gaining large sample sizes and establishing field-based trials has been difficult.

Choice-based feeding trials usually involve the presentation of food in two or more bowls to one test animal (Campbell & Bullard, 1972 described in Bullard *et al.*, 1978). The stimulus (i.e. the test article) is usually mixed in, or presented with the food of one bowl, while food in the other bowl is not treated or presented with a control substance (Nolte & Mason, 1998). The trial is then repeated on several different test subjects (Campbell & Bullard, 1972 described in Bullard *et al.*, 1978). In some situations, groups or colonies of animals are presented with the bowls (Abbott *et al.*, 1990; Avery *et al.*, 1992; Boag & Mlotkiewicz, 1994; Moran, 2001): however, this may raise issues of independence. Following the screening of multiple potential repellents, focus is often placed on one or two repellents that were most effective in preliminary trials to enable further detailed study (e.g. Montague *et al.*, 1990; Arnould & Signoret, 1993; Murray *et al.*, 2006).

Investigation into repellents for use with Australian mammals has been limited: however, promising research has been conducted with *Trichosurus vulpecula* (brushtail possum: Eason & Hickling, 1992; Morgan & Woolhouse, 1995; Woolhouse & Morgan, 1995; Cooney, 1998), *Macropus parma* (parma wallaby) and *Thylogale thetis* (red-necked pademelon: Ramp *et al.*, 2005), *Wallabia bicolor* (swamp wallaby: Montague *et al.*, 1990; Montague, 1994) and *Pteropus poliocephalus* (grey headed flying fox: Van Der Ree & Nelson, 2002 see section 1.2.2 for more detail).

Macropus rufogriseus has been identified as a problem species for agriculture and forestry in Tasmania and Victoria (Tasmanian Farmers and Graziers Association, 2004; Le Mar & McArthur, 2005; While & McArthur, 2005). Some repellents have been used in forestry management (Witt *et al.*, 2003): however, only a limited number of studies investigating repellents in Australia have been published (Johnston *et al.*, 1998; While & McArthur, 2006). *Macropus rufogriseus banksianus* is a good test specimen as its biology and ecology have been extensively studied (see Section 1.3) and large numbers of captive subjects were available to study at the University of New South Wales (UNSW) Cowan Field Station. *Macropus rufogriseus banksianus* is also a common victim of vehicle collisions in New South Wales and the results obtained from this test species will be directly relevant to the overall project objectives (Section 1.4).

2.1.2 Aims

The aim of this trial was the preliminary investigation of repellents for use with *M. rufogriseus banksianus*. It was anticipated that these pilot trials would contribute to the working knowledge of repellents and their efficacy with macropods in the Australian environment. The trial aimed to screen several repellents using standard methods to identify suitable repellents enabling further research to specifically target the role of repellents in mitigating vehicle-macropod collisions. The results of this trial may also have relevance for the Australian agriculture and forestry sectors for reducing herbivory, as studies on the effects of repellents with Australian mammals are limited.

The objectives of this captive, choice-based feeding trial were to:

1. Test the effectiveness of four repellents in reducing visitation of captive *M. rufogriseus banksianus* to feeding stations;

2. Test the effectiveness of four repellents in reducing food consumption of captive *M. rufogriseus banksianus*; and

3. Determine the most effective of the repellents, allowing further trials to focus on only one or two substances.

2.2 Methods

The trial described in this chapter received ethics approval from the Animal Care and Ethics Committee of the Director General of New South Wales Agriculture (Approval Number 02/1926) and also from the UNSW Animal Care and Ethics Committee (Approval Number 02/90). Copies of the ethics approvals and the National Parks and Wildlife Service Permit are located in Appendix A.

2.2.1 Study area

The two-choice feeding trial was conducted from August to October 2002 at the UNSW Cowan Field Station. The UNSW Cowan Field Station is located in Muogamarra Nature Reserve, near the suburb of Cowan, approximately 40 kilometres north of Sydney. As the field station is located in a nature reserve, there is very limited interaction between captive animals and people, domestic/agricultural animals, industry, agriculture and urban landscapes. Access to the station is via a locked fire trail and the nearest public road is over one kilometre away. The field station is used primarily for research, and the holding and breeding of macropods and other Australian vertebrates.

Outdoor enclosures C2, C3 and C4 (Figure 2.1) were utilised for the trial. The enclosures were adjacent and have adjoining large gates that remained open for the entire trial. Feed sheds were located in C2, C3 and C4: however, the feed shed in C3 was not used during the trial for feeding. Animals retained access to the feed shed in C3 for shelter.

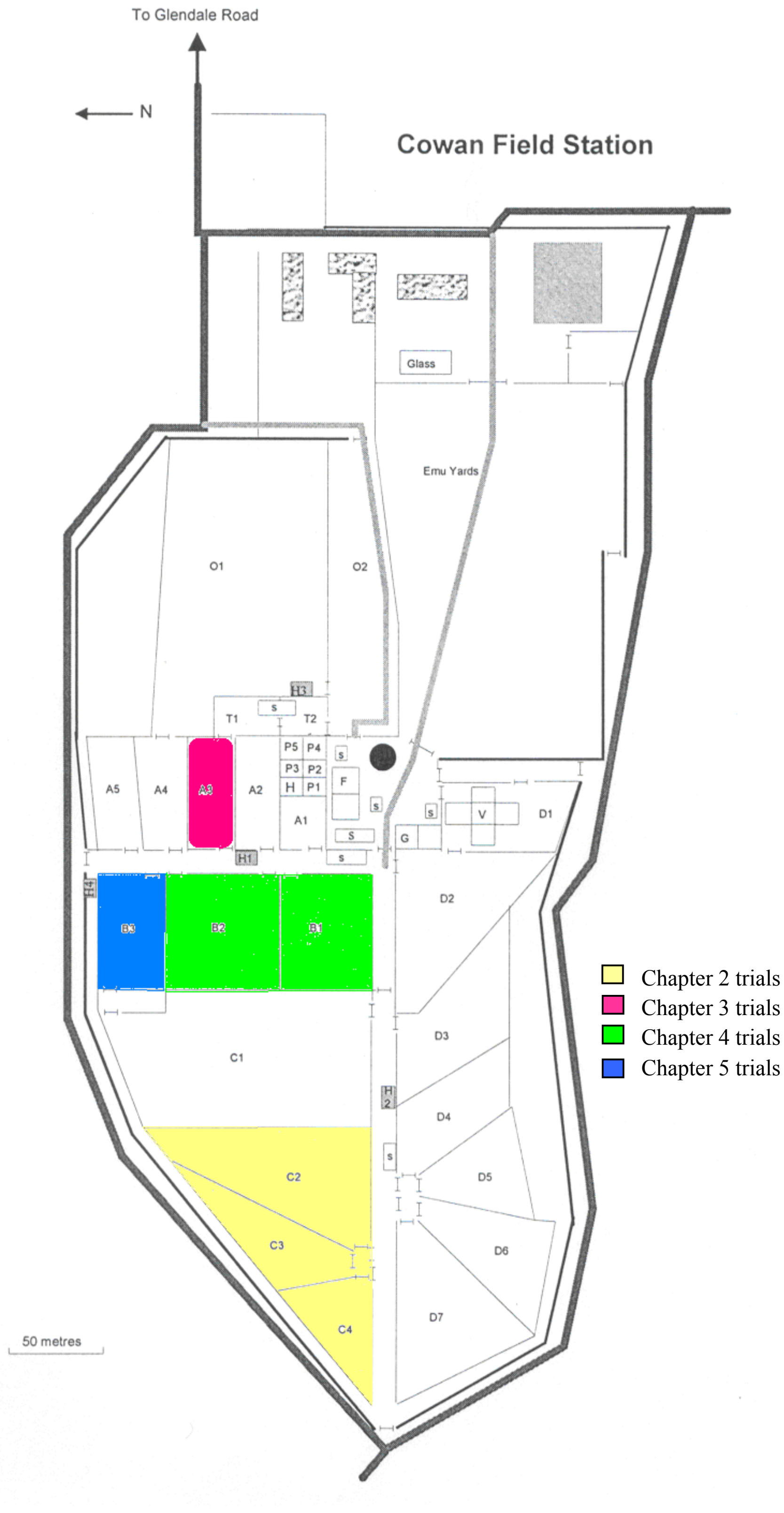

Figure 2.1 Design and layout of the University of New South Wales, Cowan Field Station (Image Courtesy of D. Croft). The trial enclosures are highlighted (see legend). The field station is located in Muogamarra Nature Reserve, New South Wales.

The enclosures were vegetated with mostly native grasses, shrubs and trees, although some exotic grass species were also present. This vegetation provided suitable habitat for *M. rufogriseus banksianus*. A similar enclosure at the UNSW Cowan Field Station has previously been described as a semi-natural environment (Hunt *et al.*, 1999). Water was available to the animals at all times via three automated watering points located in each enclosure. The watering points were cleaned regularly throughout the trial.

The feed sheds in C2 (referred to as Feed Tray A) and C4 (referred to as Feed Tray B) were the only two feeding stations used in this choice-based trial. The feeding sheds were separated by 45 m. Feed sheds and food trays were cleaned each day throughout the trial. The floors of the feed sheds were raked and the trays were washed and scrubbed (water and brush) and alternated with another set of trays to allow drying between uses. Infrared cameras (1/3" CCD, black and white camera with 4.3 mm lens, and LEDs) were located in each feed shed. The cameras were used to monitor animals while feeding and approaching the feed trays. The infrared cameras were connected to time-lapse videocassette recorders (TL VCRs) to allow the footage to be recorded (9:1 real time: recorded time).

2.2.2 Study subjects

Ten *M. rufogriseus banksianus* (3 male: 7 female) were involved in the trials. Animals belonged to a captive colony, but were not tame or habituated to human presence. Animals were not separated and remained as a group throughout the trial. Animals had no previous exposure to odour repellents as part of any experiment. Animals had been previously used in an observational trial conducted by researchers from the UNSW. The previous trial had involved exposing the animals to flashing lights and recording behavioural responses. Animals were introduced to the trial enclosure and allowed to acclimate for seven days before the commencement of the trial.

2.2.3 Repellents

Repellents were selected for this trial for the following reasons:

1) Recommendation for use by any Australian governmental body;
2) Commercially available product; and
3) Previous success in repelling marsupials documented in the scientific literature.

To reduce the number of substances to be tested, a variety of further methods of selection were utilised. For substances selected from point one (recommended for use in Australia, by a government body) the most commonly cited substance with high approval rating was selected. Most substances that were identified from point two (commercially available) contained aluminium ammonium sulphate as the active ingredient. Products containing other active ingredients were found, however the aluminium ammonium sulphate containing substances were most numerous. One of these products disseminated through a national retail chain, was selected. Three substances that had previously been reported in scientific literature as effective in repelling marsupials were selected.

The repellents selected for this trial were:

1. Plant Plus. Produced and manufactured by Roe Koh and Associates Pty. Ltd. (Mornington, Victoria), Plant Plus was formerly known as Pine Plus and TOM. It is manufactured in Australia and was previously tested on *Trichosurus vulpecula* (common brushtail possum) and *Oryctolagus cuniculus* (rabbit) in New Zealand (Morgan & Woolhouse, 1995; 1998). It is described as synthetic dog urine (Dr Thomas Montague, Roe Koh and Associates Pty. Ltd., pers. comm.). Following the completion of the trials, further work with Plant Plus and marsupials was published (Ramp *et al.*, 2005; While & McArthur, 2006). The repellence of canine urine has also

been noted in Australia (Montague *et al.*, 1990; Blumstein *et al.*, 2002; Murray *et al.*, 2006; Hayes *et al.*, 2006; Parsons *et al.*, in press) and elsewhere (e.g. Sullivan *et al.*, 1985a, 1985b; Arnould & Signoret, 1993; Nolte *et al.*, 1994a; Englehart & Muller-Schwarze, 1995; Arnould *et al.*, 1998; Rosell & Czech, 2000; Hubbard *et al.*, 2004). For this trial, Plant Plus was prepared for use by diluting with water to the concentration recommended by the manufacturer (concentration of active constituents confidential).

2. SCAT® Bird and animal repellent. Developed and manufactured in Australia by Multicrop (Pty. Ltd.), SCAT is designed to discourage pets and other animals from entering gardens and particular domestic settings. The active constituent is aluminium ammonium sulphate. The packaging directions recommend it for use with dogs, cats, birds (including ducks), rabbits, rats and possums. This product was selected due to its active ingredient and the product's wide distribution and ease of purchase. SCAT® bird and animal repellent has also been reported to be at least partially effective for repelling *T. vulpecula* (Cooney, 1998). SCAT® was prepared according to the instructions supplied by the manufacturer by diluting with water to a concentration of 50 g of aluminium ammonium sulphate per litre.

3. Egg formulation. Ready Eggs (Farm Pride Products: Keysborough, Victoria) is a commercially available, pasteurised, whole chicken egg product. The product is available in pouches for cooking purposes. The product was selected for use as an egg based repellent. Egg based repellents have been suggested as marsupial repellents by many governmental departments in Australia including: Tasmanian Parks and Wildlife Service (Parks & Wildlife Service of Tasmania, 2002); Queensland Department of Natural Resources (Officers, 1996); and the Victorian Department of Sustainability and Environment (Department of Sustainability and Environment, 2002). Ready Eggs

was not diluted for these trials and was presented in the form it was purchased (i.e. liquid mix containing 100% whole chicken egg). Following completion of the trials, Pestat Ltd. released a synthetic fermented egg formulation (SFE) packaged in an aerosol can for the specific purpose of animal control.

4. IPMS (Δ^3-isopentenyl methyl sulfide). IPMS is a constituent of *Vulpes sp*. (fox) urine (Jorgenson *et al*., 1978; Wilson *et al*., 1978) and it has been shown to be effective alone and in combination with other chemicals. It has been an effective repellent for *Microtus montanus* and *M. pennsylvanicus* (voles), *Thomomys talpoides* (pocket gophers), *Tamiasciurus hudsonicus* (squirrels) and *Trichosurus vulpecula* (Sullivan *et al*., 1988; Woolhouse & Morgan, 1995). IPMS was selected by Montague *et al.* (1990) as a potential repellent for *W. bicolor* but was not selected for field trials following poor results in preliminary trials. As IPMS is highly volatile, it was diluted in paraffin for application at a concentration of 5% weight/volume following the methods of Woolhouse & Morgan (1995).

One repellent initially selected for use, 3,3-dimethyl-1,2-dithiolane (DMDT) was not used in the trials due to difficulties encountered in the supply of chemicals required for manufacture and also difficulties in the direct purchase of the substance and importing conditions. Further trials with DMDT are recommended if availability is secured.

2.2.4 Procedure

During this pilot study, study subjects remained as a group to enable general observations of animals in a familiar test environment. This has been previously reported for pilot repellent screening studies (e.g. Boag & Mlotkiewicz, 1994; Moran, 2001) as group responses may provide additional anecdotal evidence, for the formulation of hypothesis for further appropriate testing. However, this method raises issues of statistical independence and recovery periods between testing dates were included in an attempt to mitigate the violation of independence (see Section 2.2.5).

Following the acclimation of subjects to the study area, 3.5 kg of pelleted kangaroo feed (Doust & Rabbidge, Concord West) was provided in each of the two feeding trays. A four-day pretrial period ensued and the consumption of food from each tray was monitored. The consumption of food was monitored daily by weighing and replacing food. The number of times animals approached each feed tray was also monitored through video surveillance.

Following the pretrial period, the trial period commenced. The trial period consisted of four, 24-hour tests for each of the four repellents plus four, 24-hour tests of controls (procedural control, water or paraffin). A recovery period of at least 24-hours preceded each test. The order of tests within the trial period was random.

Between 3:00 and 4:00 pm (AEST) on each day of the trial, 3.5 kg of food was placed in each feeding tray. On each test day, a petri dish was attached to each feed tray using double-sided tape (Figure 2.2). Fifteen millilitres of the treatment substance was added to the petri dish on Feed Tray A. The petri dish at Feed Tray B was filled with 15 mL of an appropriate, paired control substance. Table 2.1 contains a list of repellents and their paired control substances. Since a consistent preference in feeding to Tray A was detected in the pretrial period (refer to Section 2.3.2), the position of the treatment was always at Tray A (rendering the test findings conservative). The first hour of each treatment period was observed from an elevated hide to assess if any animals showed signs of distress (e.g. rapid flight). If distress was noted, treatments were immediately removed and subjects monitored to assess if further action was necessary.

Consumption of pelleted food was calculated for each feeding tray (mass of food removed) by weighing the amount of food remaining in each tray after each test. The number of times *M. rufogriseus banksianus* approached the feeding trays over the 24-hour period was monitored and scored as “head dips”. An approach was defined as “a *M. rufogriseus banksianus* placing any portion of its head below the rim of the feed tray”. This could be determined objectively from visual analysis of the video surveillance.

The use of both of these variables was necessary as non-target species (including various species of birds, rats and possums) could gain access to the trial arena and had the potential to confound consumption as a variable for the target species. The approach variable was specific to the target species, however the consumption data were retained and used (with limitations) to assess feeding deterrence.

Control substances (water, paraffin) were tested using the same methods with the exception of being paired with an empty petri dish (Table 2.1). A procedural control was also assessed by the same methods with the exception that an empty petri dish was attached to Tray A, while Tray B remained free of a petri dish. Following the completion of the two-choice feeding trials, animals were returned to the care of the staff of the UNSW Cowan Field Station and carefully monitored.

Figure 2.2 Photograph of feed station with empty petri dish attached to centre of feed tray.

Table 2.1 Treatment and control substances used for the captive two-choice feeding trial and the number of test days.

Treatment	Paired Control	Number of tests
Plant Plus	Water	4 test days
SCAT	Water	4 test days
Putrescent egg	Water	4 test days
IPMS	Paraffin	4 test days
Water	Empty petri dish	4 test days
Paraffin	Empty petri dish	4 test days
Procedural Control (Empty petri dish)	No Petri dish	4 test days
Recovery period following each test day (Nothing attached to feed tray)		33 days
Acclimation		7 days
Pretrial monitoring		4 days
Total days		**80 days**

2.2.5 Data analysis

The mass of food consumed from (mass), and the number of approaches to (head dips by *M. rufogriseus banksianus*) each feed tray were the main dependent variables analysed for this trial. Treatment was the independent variable. The recovery periods between each trial were intended to retain independence between tests. However, as each test was performed on the same set of subjects, a violation of independence occurred. To adjust for the violation of independence, a conservative level of significance (alpha = 0.01) was used for statistical analyses. This adjustment is recommended for mild violations of independence by Stevens (2002).

To indicate feeding preference, the difference between treated and untreated tray was calculated to determine which feeding tray received the most activity for each test. Two new variables were calculated: Consumption (mass of food) difference; and Approach (head dips) difference. The variables were calculated following the difference score method described by Nolte & Mason (1998). This was performed by subtracting the Tray B variable from the comparative Tray A variable. For the new consumption difference variable (dif mass) a positive value indicates that more food was consumed (mass) from Tray A, with the magnitude of the difference being directly represented by the value. A negative value indicates more food being consumed from Tray B, with the value representing the magnitude of difference. For the new approach difference variable (dif dips), a positive value indicates a greater number of approaches to Tray A compared to Tray B, with the value indicating the magnitude of the difference. A negative value indicates more approaches to Tray B than Tray A.

Statistical Analysis was performed using SPSS 13.0 for Windows (SPSS Inc, 2004). Data were screened for outliers, errors and normality. Correlations were performed with dependant variables to assess strength of theoretical relationships (if choice exists an inverse relationship would be expected). Total consumption of food (Tray A plus Tray B) was analysed to assess if there were any changes in the amount of food consumed by *M. rufogriseus banksianus* between treatments during the trial that may have produced unintentional effects on variables and reduced independence. Similarly, the total number of approaches (to Tray A plus to Tray B) was analysed for any differences.

Comparisons of each preference variable were made utilising Analysis of Variance (ANOVA) techniques with *a priori* contrasts testing specific hypotheses. General Linear Modelling and Multivariate analyses were not performed due to violations of homogeneity of variance-covariance matrices and multicollinearity assumptions. Levene's test for homogeneity of variance was used to screen for violations of the homogeneity of variance assumption. When mild heterogeneous variance occurred (Levene's value with $0.01<p<0.05$), a Brown-Forsythe corrected ANOVA was used. With more severe violations of homogeneity of variance (Levene's test value with $p<0.01$), non-parametric analysis was performed (Kruskal-Wallis test). The same methods were used to analysis each of the four basic variables (consumption from Tray A, consumption from Tray B, approaches to Tray A and approaches to Tray B). These data are presented in Appendix D.

2.3 Results

2.3.1 Data Screening

Data were screened by examination of summary statistics for each variable, separated into treatment groups. Summary statistics, boxplots and normality tests (Shapiro-Wilk) indicated that most data sets were approximately normal. An exception was for the egg formulation where several variables returned a significant Shapiro-Wilk value (Table 2.2).

Table 2.2 Normality test results for all variables for the egg formulation. All variables of other treatments and controls returned non significant normality tests. Significant results are highlighted.

	Shapiro-Wilk Statistic	Significance
Consumption (grams) from Tray A	0.72	<0.05
Consumption (grams) from Tray B	0.92	>0.05
Approaches (head dips) to Tray A	0.85	>0.05
Approaches (head dips) to Tray A	0.86	>0.05
Consumption difference	0.76	<0.05
Approach difference	0.71	<0.02

An outlier was detected for multiple variables from the Egg treatment group. Following consultation with field notes taken on the day when the outlier occurred (13 October 2002), the data from this day was removed. This was due to the nature of the data and a note in the field book stating that the egg formulation used on 13 October 2002 had been stored incorrectly, and as a result smelt and looked differently and contained insect larvae. The removal of this sample resulted in a reduced sample size of three for the egg treatment. However, the data set was now considered close to normal (consumption from Tray A was still detected by Shapiro-Wilk tests: 0.75, $p<0.01$). All other data sets remained intact with each treatment group maintaining a sample size of four.

No significant difference in the total amount of food consumed by subjects (sum of mass of food consumed from both feed stations for each test) was found between treatment groups

$F[7,23]=1.45$, $p>0.01$). Additionally, no difference was detected between treatments for the total number of approaches to both feed trays ($F[7,23]=1.67$, $p>0.01$) indicating a consistence in feeding responses throughout the trial period.

The mass of food consumed from Tray A was inversely related to the mass of food consumed from Tray B ($r=-0.78$, $n=31$, $p<0.0005$) supporting the existence of choice in the trial. Similarly, the number of approaches to Tray A was also inversely correlated with the number of approaches to Tray B ($r=-0.92$, $n=31$, $p<0.0005$). Additionally, there was a correlation between consumption difference (dif mass) and approach difference (dif dips) with a strong positive direction ($r=0.96$, $n=31$, $p<0.0005$).

2.3.2 Pretrial preference

A paired samples t-test on the mass of food consumed from the two feed trays during the pretrial stage revealed that subjects displayed a consistent preference to feed from Tray A located in enclosure C3 ($T=9.339$, $p<0.005$). The mean mass of food consumed from Feed Tray A was 60% greater than the mean mass of food consumed from Feed Tray B (3313±113 g and 2063±90 g respectively). A similar preference was detected from the video surveillance data with the mean number of approaches (head dips) by *M. rufogriseus banksianus* to Feed Tray A significantly greater than the mean number of approaches to Feed Tray B ($T=8.047$, $p=0.004$). The mean number of approaches to Feed Tray A was approximately 2.5 times the mean number of approaches to Feed Tray B (1230±56 and 475±51 approaches respectively). Due to theses results, all repellent substances were placed at Feed Tray A, rendering tests of repellent efficacy conservative (see Section 2.2.4).

2.3.3 Trial results

For both consumption of food (mass) and approaches (head dips), tray preference was calculated by subtracting the Tray B variable from the Tray A variable (refer to Section

2.2.5). Figure 2.3 displays the consumption (mass) difference data for each treatment. A significant effect on the consumption difference score was observed with treatment ($F[7,23]=22.09$, $p<0.0005$, eta squared=0.87). A series of *a priori* contrast were performed (Table 2.3). No difference in tray preference in mass of food consumed was detected between the pretrial (M=1250) and procedural control (M=1325), water (M=1313) and paraffin (M=1125) groups. A significant difference in preference was found between the Plant Plus (M=-1875) and water, and also egg (M=-1983) and water. There was no significant difference in food preference (mass) between SCAT (M=1425) and water. A result approaching significance was detected between IPMS (M=150) and paraffin.

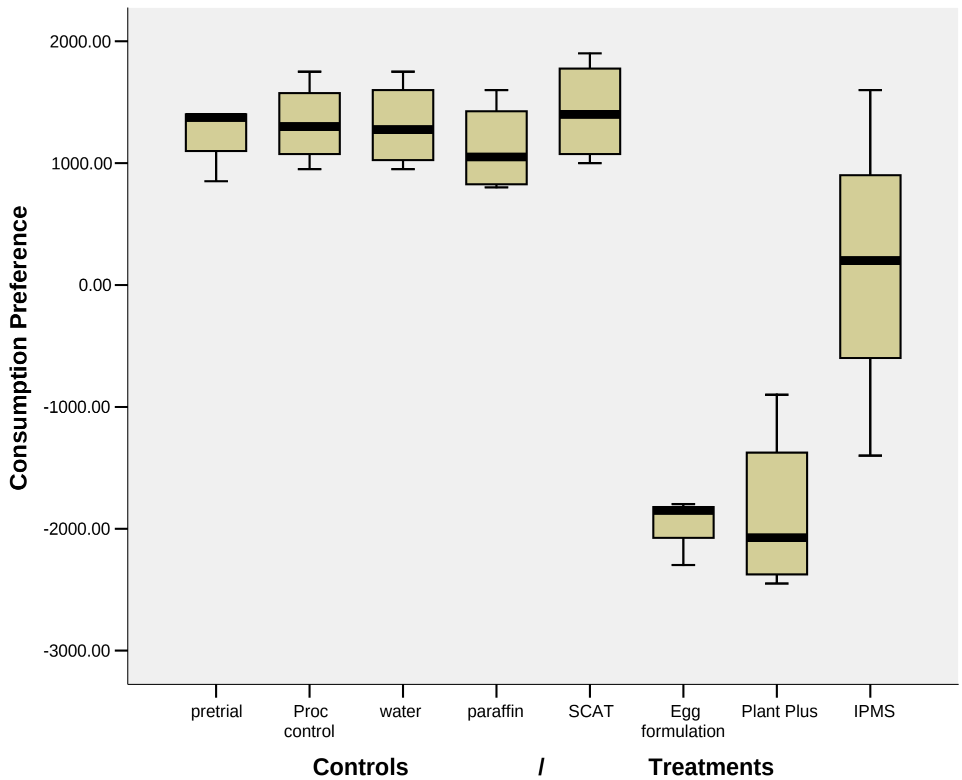

Figure 2.3 Preference in consumption (g) to each tray. A positive value indicates that more food was consumed from Tray A. A negative value indicates that more food was consumed from Tray B (Consumption Preference = mass of food consumed from Tray A minus mass of food consumed from Tray B). A one-way ANOVA detected a significant effect between treatments ($F[7,23]=22.09$, $p<0.0005$, eta squared=0.87).

Table 2.3 Consumption preference -a series of *a priori* contrasts test specific hypotheses. Each row represents a contrast. Contrast 1 is two-tailed, contrasts 2-5 are one-tailed. Alpha = 0.01. Significant results are highlighted.

Contrasts: Consumption preference												
	Contrast Coefficients								Result			
	Pretrial	**Procedural Control**	**Water**	**Paraffin**	**SCAT**	**Egg**	**Plant Plus**	**IPMS**	**F**	**df 1**	**df 2**	**p**
1	-3	1	1	1	0	0	0	0	0.00	1	23	>0.99
2	0	0	1	0	0	0	-1	0	58.25	1	23	<0.0005
3	0	0	1	0	0	-1	0	0	53.38	1	23	<0.0005
4	0	0	1	0	-1	0	0	0	0.07	1	23	>0.39
5	0	0	0	1	0	0	0	-1	5.45	1	23	0.015

Macropus rufogriseus banksianus approach preferences are displayed in Figure 2.4. Heterogeneity of variance resulted in the utilisation of a non-parametric Kruskal Wallis test. A significant difference was detected between treatments for approach preference (χ^2_7=19.18, p<0.008). A series of *a priori* contrasts were performed (Table 2.4). There was no significant difference in approach preferences between the pretrial (M=755) and the procedural control (M=560), water (M=722) and paraffin (M=567) groups. Preference was significantly different between the water group and both the Plant Plus (M=-1586) and Egg (M=-1335) treatments. There were no significant differences between water and SCAT (M=826) or IPMS (M=151) and paraffin.

Table 2.4 Approach preference - A series of *a priori* contrasts were run to test specific hypotheses. Each row represents a contrast. Contrast 1 is two-tailed, contrasts 2-5 are one-tailed. Alpha = 0.01. Significant results are highlighted.

Contrasts: Approach preference												
	Contrast Coefficients								Result			
	Pretrial	**Procedural Control**	**Water**	**Paraffin**	**SCAT**	**Egg**	**Plant Plus**	**IPMS**	**F**	**df 1**	**df 2**	**p**
1	-3	1	1	1	0	0	0	0	1.78	1	4.4	>0.24
2	0	0	1	0	0	0	-1	0	242.15	1	4.5	<0.0005
3	0	0	1	0	0	-1	0	0	577.06	1	5.0	<0.0005
4	0	0	1	0	-1	0	0	0	0.28	1	3.8	>0.31
5	0	0	0	1	0	0	0	-1	0.97	1	3.0	>0.19

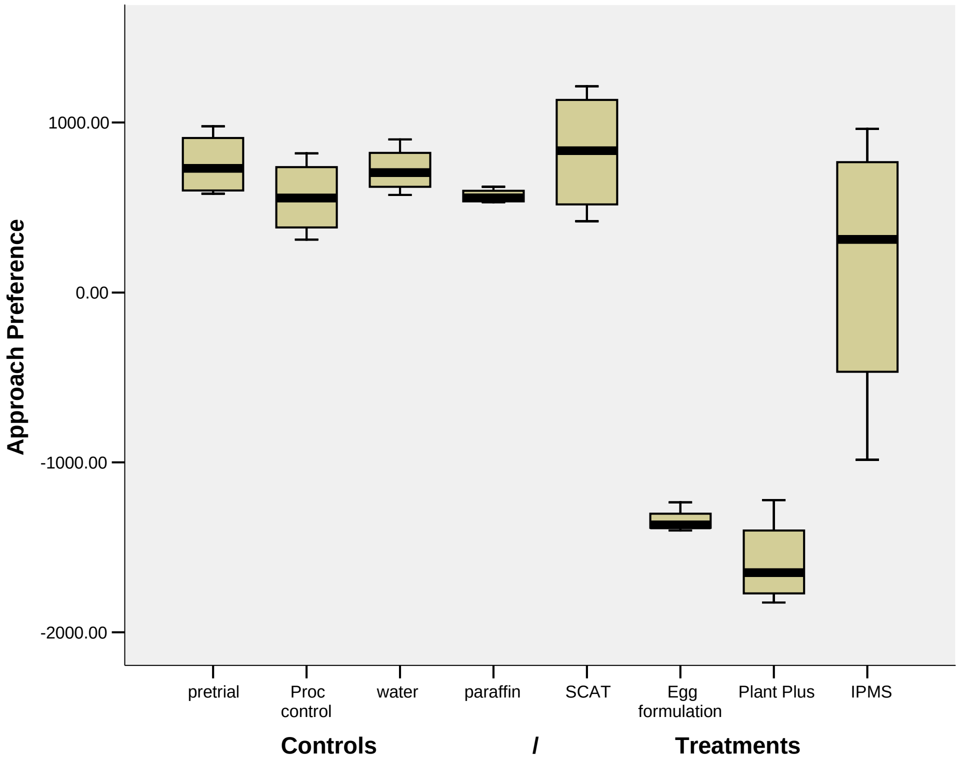

Figure 2.4 Tray preference in approaches by *M. rufogriseus banksianus*. A positive value indicates that more approaches were made to Tray A. A negative value indicates that more approaches were made to Tray B (Approach preference = number of approaches to Tray A minus number of approaches to Tray B). A Kruskal Wallis test indicated that treatment had a significant effect (χ^2_7 =19.18, $p<0.008$).

2.4 Discussion

Both Plant Plus and the egg formulation significantly affected feeding and approach preference for *M. rufogriseus banksianus*. Avoidance by *M. rufogriseus banksianus* of feed stations with Plant Plus and egg was clearly evident and significant reductions in food consumption and numbers of approaches to trays were observed. IPMS may have induced a weak response as a change in preference of consumption nearing significance was detected (Figure 2.3). However, no change in the approach preference or the raw approach or consumption data was detected for IPMS. SCAT® Bird and animal repellent did not alter feeding or approaches to feed stations for *M. rufogriseus banksianus* and achieved similar results to the control procedures.

The presence of *Rattus norvegicus* and *Trichosurus vulpecula* in the trial arena confounded consumption (mass of food) as a variable for assessing the effects of the repellents on *M. rufogriseus banksianus*. The effect of this confound is likely to be small, since the consumption and approach indices were highly correlated (r=0.96). However, the method used to calculate the approach variable (i.e. observation of target species) removed the confounding factors.

The magnitude of the difference between the controls and the Plant Plus and egg treatments was notably large, with approximately 1000 fewer approaches to feed stations per day for the Plant Plus and egg treatments (Appendix D). This suggested that Plant Plus and egg treatments were good repellents for *M. rufogriseus banksianus* even though animals were not totally repelled.

A preference to feed from Tray A by test subjects was detected before the start of the trial phase. The reason for the significant preference in feeding was not determined, but could be related to differences in the vegetation and topography in the immediate vicinity of the feed stations. A distance of 45 m separated the feed stations: however, Tray A was located on higher, uneven ground, with more grasses in the immediate vicinity of the feed station. Tray B was located on a very flat section of the enclosure and had more leaf litter covering the ground with less vegetative ground cover. Distances to alternative food sources or shelter are likely reasons for the preference.

Due to the pre-trial preference, the placement of treatments was not random and all treatments were placed at Tray A. This was done to reduce the potentially confounding influence of the pre-existing preference and to assess if the repellents invoked an aversion that was stronger than the pre-trial preference for Tray A. Testing all repellents at the one feed station could potentially lead to conditioned aversion of the feed tray (see Garcia *et al.*, 1955): however, the recovery periods were intended to reduce this potential and the low variance in all control groups indicated that conditioned aversion was unlikely.

Ramp *et al*. (2005) also detected the repellent qualities of Plant Plus with macropods when investigating vigilance and proximity responses of *T. thetis* and *M. parma*. *Macropus parma* reduced contact (time spent in proximity) to Plant Plus. The results observed in this study with *M. rufogriseus banksianus* were similar to those obtained for *M. parma* by Ramp *et al*. (2005). However, the response of *T. thetis* to Plant Plus differed to the responses of *M. parma* and *M. rufogriseus banksianus*, and more time was spent in proximity to Plant Plus in an increased state of vigilance. Ramp *et al*. (2005) identified the response of both *T. thetis* and *M. Parma* as being defensive and related the differences in response to differing anti-predator strategies.

Two previous formulations of Plant Plus, labelled TOM and Pine Plus[1], have been trialled in New Zealand to assess whether they could reduce browsing damage to pine trees caused by *T. vulpecula* and *O. cuniculus* (Morgan & Woolhouse, 1995; 1998). The earliest formulation (TOM) significantly reduced browsing by both *T. vulpecula* and *O. cuniculus* in captive and field trials but displayed phytotoxic properties and was not suitable for use on seedlings (Morgan & Woolhouse, 1995). The updated formulation (Pine Plus) was also effective at reducing browsing by *T. vulpecula* and *O. cuniculus* but was not phytotoxic and was recommended as a treatment for forestry seedlings (Morgan & Woolhouse, 1998). The results of this study support the findings of Morgan & Woolhouse (1995; 1998) and Plant Plus may be suitable for forestry purposes in Australia since *T. vulpecula*, *O. cuniculus* and several species of macropod (including *M. rufogriseus rufogriseus*: Bennett's wallaby, *T. billardierii*: Tasmanian pademelon and *W. bicolor*) cause damage to plantations through herbivory (Montague *et al.*, 1990; Bulinski & McArthur, 1999; McArthur *et al.*, 2000; 2003).

Plant Plus is a formulation (composition confidential) based on dog urine but contains additives to increase its longevity (Dr Thomas Montague, Roe Koh and Associates Pty. Ltd., pers. comm.). Dog urine was effective in reducing damage to *E. regnans* caused by browsing of *W. bicolor* in both captive and field trials (Montague *et al.*, 1990) and also induced a flight response in *M. fuliginosus* (western grey kangaroo: Parsons *et al.*, in press). It was proposed that the effectiveness of the dog urine was related to a fear response in the test subjects. Ramp *et al.* (2005) also suggested that macropod responses to Plant Plus were anti-predator strategies and Blumstein *et al.* (2002) proposed that macropods with predator experience can respond with anti-predator strategies to predator odours. However, the results of this trial and the results obtained by Ramp *et al.* (2005) are contrary to the theory of Blumstein *et al.* (2002)

[1] The active ingredients of TOM, Pine Plus and Plant Plus were the same, the main difference between the formulations was the ingredients used to adhere the repellent together and aid in the application to substrate (Dr Thomas Montague, Roe Koh and Associates Pty Ltd, pers. comm.).

in that predator naïve macropods were successfully repelled, presumably as a result of a fear response.

Preliminary screening of repellents for *W. bicolor* by Montague *et al.* (1990) included a trial of chicken eggs as well as synthetic fermented egg. Synthetic fermented egg, and natural egg products have been successful at repelling a range of herbivores and attracting carnivorous mammals (Bullard *et al.*, 1978). However, in contrast to the egg formulation used for this trial, neither egg or synthetic fermented egg significantly reduced browsing damage to *E. regnans* in the short-term captive trials with *W. bicolor* (Montague *et al.*, 1990). The potential reasons for the discrepancies between the results achieved in this trial for egg and the results obtained by Montague *et al.* (1990) are many and include differences in methods and species utilised. Synthetic fermented egg has been effective with *T. vulpecula* in New Zealand (Woolhouse & Morgan, 1995) and non fermented egg has also shown promise with *T. vulpecula* (Eason & Hickling, 1992). Egg has been identified as an effective short term repellent for several other herbivores including *Cervus elaphus nelsoni* (elk: Andelt *et al.*, 1992) and *Odocoileus spp*. (deer: Palmer *et al.*, 1983; Andelt *et al.*, 1991) and several effective deer repellents are based on compounds found in chicken eggs (e.g. MGK Big Game Repellent® and Deer Away®, see Melchiors & Leslie, 1985; White & Blackwell, 2003 and refer to Appendix C). In spite of the negative results of egg as a repellent for *W. bicolor* (Montague *et al.*, 1990), the results of this trial indicate that egg could be an effective repellent for macropods and should be further investigated.

IPMS has been trialled as a repellent for several species with mixed results (Lindgren *et al.*, 1995). The inability of IPMS to produce a significant reduction in feeding and approaches by *M. rufogriseus banksianus* was similar to the lack of response by *W. bicolor* reported by Montague *et al.* (1990). Conversely, IPMS has been effective in significantly reducing browsing damage in captive trials with *T. vulpecula* (Woolhouse & Morgan, 1995). IPMS has also been shown to reduce feeding in *Lepus americanus* (snowshoe hares: Sullivan & Crump, 1986). Due to the preliminary nature of this trial and some ambiguous results for IPMS, it is not possible to dismiss IPMS as a repellent for macropods. However, its effects with *M. rufogriseus banksianus* were not as strong as those of Plant Plus or egg and further work is needed to establish its repellent qualities.

SCAT® Bird and animal repellent was ineffective in these trials, despite displaying repellent properties when tested with *T. vulpecula* (Cooney, 1998). The composition of SCAT® Bird and animal repellent (>99% Aluminium Ammonium Sulphate undiluted: 50 g/L when diluted for use following manufacture's instructions: Multicrop, 2003) is similar to many other commercial repellents and the results obtained in this trial are most likely applicable to all repellents that are based on aluminium ammonium sulphate.

The primary limitation of this trial was the violation of the assumption of independence associated with the statistical tests. This violation was partially addressed by incorporating recovery periods between trial days and decreasing the significance level of statistical tests (α=0.01). A more stringent approach to addressing this violation would be through the establishment of the extinction of response rates for each treatment in a series of preliminary trials (see section 2.3 of Takahashi *et al.*, 2005). However, the establishment of extinction rates would be time and resource intensive and would counter the purpose of this trial as a pilot study.

The visitation of non-target animals to the trial arena (specifically to the feed stations) is also a limitation of the trial as non-target animals had the potential to affect the behaviour of the target species. Plant Plus was reported to be an effective repellent for use with one of the non-target species (*T. vulpecula*: Morgan & Woolhouse 1995; 1998); however, visitation of the non-target species was observed at both feed stations on all trial days. Additionally the objective of this research was to identify suitable repellent/s for environmental application and non-target species will invariably be present in any application. For these reasons, the presence of non-target species in the trial arena was unlikely to have introduced an unacceptable level of error in consideration of the objectives of this trial.

The choice-trial format successfully identified the most effective repellent substances and enables further work to focus on only Plant Plus and the egg formulation. The sensitivity of the trials was limited due to methodological constraints (independence, number of subjects, captive environment) and further work is necessary to identify the repellent properties of Plant Plus and the egg formulation. Future work with DMDT is also suggested if a suitable supply source can be identified. IPMS may have some repellent qualities for use with *M. rufogriseus banksianus*, however further elucidation of these is required and the response observed in this trial was not as strong as those detected for Plant Plus and egg. SCAT® Bird and animal repellent does not show promise as a repellent for *M. rufogriseus banksianus*.

Chapter 3

Movement of *Macropus rufogriseus banksianus* through a scent barrier

Chapter 3 Movement of *Macropus rufogriseus banksianus* through a scent barrier

3.1 Introduction

3.1.1 Background

Mammal repellents generally work by: inducing fear; conditioned aversion; pain; or taste (Beauchamp, 1995; Wagner & Nolte, 2001). The majority of fear-based mammal repellents are sulfurous compounds and are usually predator odours (or derivatives). Predator odours have been shown to reduce locomotor activity and non-defensive behaviours in captive studies (see Apfelbach *et al.*, 2005). In field studies, three behaviours in response to predator odours have been intensively studied and could be used effectively for wildlife management. These include: changes in activity patterns; reduction in non-defensive behaviours (grooming, feeding, reproducing); and habitat shifts (reviewed by Apfelbach *et al.*, 2005).

Repellents that utilise conditioned aversion rely on the target species forming an association between the treated substance and an unpleasant sensation (Wagner & Nolte, 2001). The unpleasant sensation might be fear, pain or taste, which may have additional repellent properties, but other reactions like illness and gastrointestinal upset are also utilised. Brown *et al.* (2000) successfully trialled lithium chloride as a repellent for *Rangifer tarandus* (caribou). It was envisaged that LiCl could be mixed with road de-icing salts to reduce the amount of time caribou spend on roads licking salt. Lithium chloride is a gastrointestinal toxicant that has also been used to condition taste aversion in domestic ruminants (Du Toit *et al.*, 1991; Ralphs & Olsen, 1992).

Trigeminal irritants are common pain-inducing repellents that have been extensively trialled (Andelt *et al.*, 1994; Baker *et al.*, 1999; Wagner & Nolte, 2000; Santilli *et al.*, 2004). Trigeminal irritants are detected by free nerve endings in the mouth and nose, and mucous membranes including the eyes and gut lining (Mason *et al.*, 1992; Nolte & Mason, 1998).

Capsaicin is the most widely tested trigeminal irritant and its properties and effects on a range of species are well documented (see Monsereenusorn *et al.*, 1982).

Denatonium benzoate (Bitrex) is a common taste repellent used in several commercial preparations (e.g. Anipel, Ropel®, Tree Guard®). Denatonium benzoate has been cited as the "bitterest tasting substance known" (Santilli *et al.*, 2004), however, results of experiments with denatonium benzoate have been inconsistent (Montague *et al.*, 1990; Swihart & Conover, 1990; Andelt *et al.*, 1991; Andelt *et al.*, 1994; Montague, 1994; Nolte *et al.*, 1994b; Witmer *et al.*, 1998; Santilli *et al.*, 2004).

Further to the categorisation of repellents into the four modes of action (fear, conditioned aversion, pain and taste), repellents can be divided by the mode of application – systemic, topical or area (Nolte, 2003). Systemic repellents are absorbed into a plant and translocated by natural internal processes. Systemic repellents are not common as efficacy has been poor (e.g. Moser, 2003) or the repellents have had adverse effects on vegetation (Nolte, 2003). Topical repellents (also referred to as contact repellents) require application to every surface in need of protection and can reduce feeding or utilisation of specific items. Area repellents are detected by target animals from a distance. In addition to achieving the same results as topical repellents, area repellents could also repel animals from target areas, prevent movement of animals into specific areas and reduce densities of target animals in preferred habitats (Seamans *et al.*, 2002).

There are several ways in which repellents could be used to reduce vehicle-macropod collisions in New South Wales. The most promising option would be the use of repellents to reduce macropod densities within road easements. This could be achieved by using an effective area repellent, or by reducing the palatability of resources (grass, water) using systemic, topical or area repellents. Decreasing macropod movements across roads by the construction of a scent-fence (as described for deer by Kerzel & Kirchberger, 1993 in Putman,

1997) or increasing vigilance behaviour (utilising a fear-inducing repellent) may also be effective in reducing macropod-vehicle collisions. However, a lack of data on the effectiveness of area repellents with macropods has prevented their use to date.

3.1.2 Aims

Plant Plus and an egg formulation were identified in Chapter 2 as having the most potential for use with *Macropus rufogriseus banksianus*. Both repellents are sulfur-based substances, with area repellent properties, with a presumed mode of action of fear. The objective of this barrier trial was to establish if Plant Plus and egg can effectively reduce movements of *M. rufogriseus banksianus* through a runway, further establishing the repellent properties. The knowledge gained from this trial would aid in the understanding of how Plant Plus and egg could be applied in the management of macropod-vehicle collisions.

3.2 Methods

The trial described in this chapter received ethics approval from the Animal Care and Ethics Committee of the Director General of New South Wales Agriculture (Approval Number 02/1926) and also from the University of New South Wales (UNSW) Animal Care and Ethics Committee (Approval Number 02/90). The trial was designed to conform to the principles outlined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH&MRC, 2004) and relevant legislation that relates to the use of animals for scientific purposes in NSW. Copies of the ethics approvals and National Parks and Wildlife service permits are located in Appendix A.

3.2.1 Study Area

The barrier trial was conducted from March to May 2003 at the UNSW Cowan Field Station (see Section 2.2.1 for details of field station). The trial was delayed from its initial commencement date of December 2002, following a serious bushfire in December 2002 that affected provision of facilities at the field station. Enclosure A3 (Figure 2.1) was utilised for this trial. A self-filling water trough was located in the centre of the yard and was accessible at all times throughout the trial. A covered feed shed was located at one end of the yard and could only be accessed from the pen through a linear fenced corridor (Figure 3.1). Native and exotic grasses provided groundcover and a variety of native trees were present. The enclosure could be described as semi-natural, and was suited to *M. rufogriseus banksianus*.

Two infrared cameras (1/3” CCD, black and white camera with 4.3 mm lens, and LEDs) were located at the entrance to the feed shed. One camera was directed towards the feed tray and monitored feeding. The other camera was directed away from the feed shed, toward the end of the linear fenced corridor. The cameras were used to monitor animals while feeding and approaching the feed shed through the linear corridor. The infrared cameras were connected to a time-lapse videocassette recorder (TL VCRs) through a quad box, allowing footage from both cameras to be recorded on one tape (9:1 real time: recorded time).

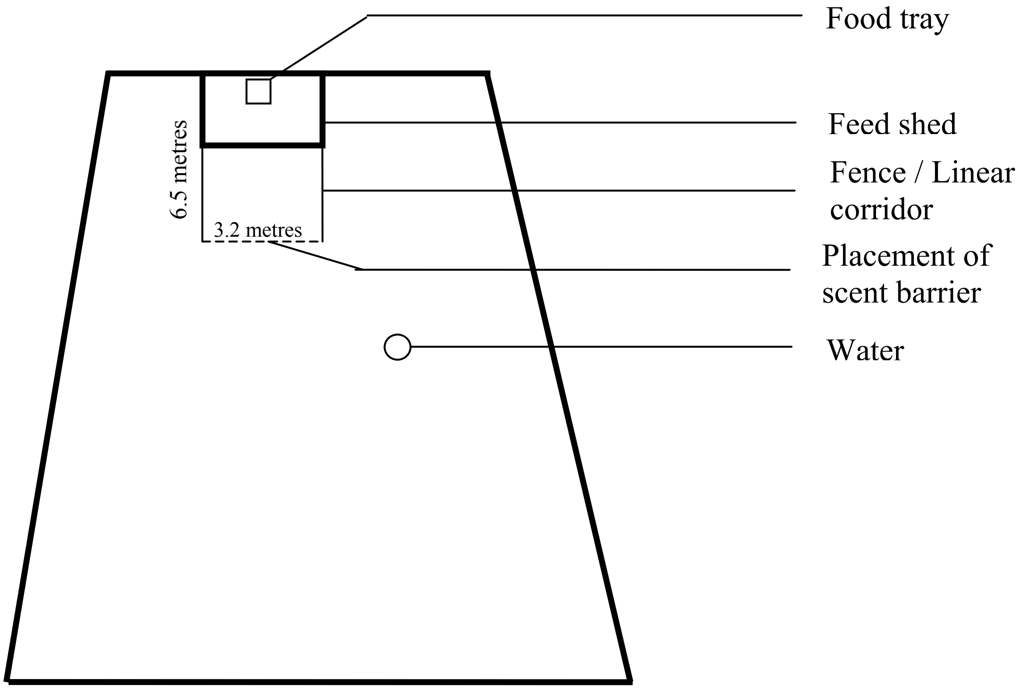

Figure 3.1 Outline of enclosure A3 displaying the placement of food, water, linear corridor and position of scent barrier.

3.2.2 Study Subjects

Ten adult *M. rufogriseus banksianus* (8 female: 2 male) were involved in the study. Animals were from a captive colony (see Section 2.2.2) and had not been previously involved in any odour related experiments. Animals were not separated and remained as a group throughout the trial. Animals were introduced to the trial enclosure and allowed to acclimate for seven days before the commencement of the trial.

3.2.3 Procedure

Four different barriers were assessed for their abilities in reducing movements of *M. rufogriseus banksianus*. To avoid permanent contamination of the enclosure, the artificial scent barriers consisted of a strip of plastic tarpaulin 3200-mm long and 300-mm wide. The tarpaulin was laid flat and tied down at the end of the linear corridor furthest from the feed area (approximately 6.5 m from the food tray). Three substances (60 mL of Plant Plus: Section 2.2.3, 60 mL egg formulation: Section 2.2.3, and 60 mL of reverse osmosis water: wet control) were lightly sprayed onto the tarpaulin, across the entire length, but kept away from the edges. A light covering of straw was then placed over the tarpaulin. A fourth treatment (tarpaulin and straw only) acted as an additional control (dry control).

Scent fences were assessed individually for 24-hour periods, commencing at approximately 3:00pm AEST. Scent fences were disposed of carefully after each 24-hour period. Each type of scent fence was assessed on four separate occasions. Recovery periods of at least 24-hours preceded each trial and the order of treatments was randomly selected.

The number of movements by *M. rufogriseus banksianus* through the linear corridor (crossing the scent fence) was calculated from video surveillance. The first hour of each trial was visually assessed from an elevated hide to assess if animals showed signs of distress (e.g. rapid flight).

Incentive to move through the linear corridor and through the scent fence was the location and provision of food. Four kilograms of pelleted kangaroo food (Doust and Babbage, Concord West) was placed in the food tray every day. Feeding areas were cleaned and food was replaced daily. Following the barrier trial, animals were returned to the care of UNSW field staff and monitored.

3.2.3 Data Analysis

General Linear Modelling (GLM) was utilised in the analysis of data for this trial (SPSS 13.0 for Windows, SPSS Inc 2004). Treatment was the fixed factor and movements past the barrier was the dependent variable. Data were screened to ensure all assumptions were met. Univariate parametric analyses, including *a priori* contrasts were also performed. During video analysis, the number of movements through the linear corridor was collated in four time frames (3pm-8pm; 8pm-midnight; midnight-6am; and 6am to 3pm). While the total number of movements was used in most analyses, the breakdown into the timeframes was used to further the understanding of trends. Recovery periods were designed to retain independence of sampling. However, a violation of independence occurred as only one group of subjects was utilised in the trial. Due to the available levels of replication, alpha levels were not reduced, but caution was exercised in interpretation of results.

3.3 Results

Data were screened by the study of summary statistics for the total movement data, divided into treatment groups. Summary statistics, boxplots and normality tests (Shapiro-Wilk) indicated the data sets were approximately normal. However, an outlier in the dry control group was detected (trial date 21 March 2003). On consultation with field notes, it was revealed that this was the first day of the trials and a note it the field book on the observations made in the first hour of the trial states animals were "unusually scared of plastic sheet". Notes taken during video analysis reveal that the tarpaulin was moving due to wind and continued moving into the evening and the feeding related behaviour was "strange". As such the number of movements through the barrier was low. Due to small sample sizes it was preferable to retain the outlier, decreasing the likelihood of type 1 error, but careful assessment of results was necessary.

The outcome of the GLM F-test using the type III Sum of Squares was significant ($F= 3.9$, $p<0.05$). The observed power of the test was high and partial eta squared was 0.49. *A priori* contrasts were conducted, testing the hypothesis that mean movements (24 h) through the treatment scent barriers (Plant Plus M=123 and egg M=142) were less than movements through control barriers (Dry control M=134 and Wet control M=158: Table 3.1). One-tail contrasts between the two control groups and Plant Plus and also the wet control and Plant Plus revealed that a significant reduction in movements past the barriers for Plant Plus were detected (Table 3.1). Movements past the egg barrier were not significantly fewer than the controls.

Table 3.1 Movement of *M. rufogriseus banksianus* past barriers: *a priori* contrasts. Significant results are highlighted.

	Contrast Coefficients				**Results**			
	Dry Control	Wet Control	Plant Plus	Egg formulation	**F**	**df 1**	**df 2**	**p**
1	1	1	-2	0	8.87	1	12	<0.03
2	0	1	-1	0	12.46	1	12	<0.006
3	1	1	0	-2	0.65	1	12	>0.05
4	0	1	0	-1	2.55	1	12	>0.05

The mean number of total movements through the Plant Plus barrier was lower than all other means (Table 3.2). This was also true for all time frames, with the exception of the 8pm-midnight section where the dry control and egg treatments were slightly lower. General linear modelling with treatment as the fixed factor and the number of movements through the barrier for each time frame as a dependent variable revealed that in the first time frame, a significant difference between treatments occurred ($F=5.02$, $p<0.02$). *A priori* contrasts revealed that movements through the Plant Plus barrier were significantly fewer than controls ($p<0.005$). No other differences between treatments were apparent and no significant results were detected for any other timeframe.

Table 3.2 Mean number of movements of *M. rufogriseus banksianus* past scent barriers. Numbers in brackets indicate mean and standard error when an outlier was excluded from the analysis.

Treatment	**Mean number of movements past scent barrier**				**Mean movements (24hr)**	**Standard error**
	Start-8pm	**8pm-midnight**	**Midnight-6am**	**6am- end**		
Dry Control	50 (53)	25 (26)	33 (35)	26 (27)	134 (141)	7.6 (5.8)
Wet Control	52	36	36	34	158	6.2
Plant Plus	39	29	32	24	123	6.7
Egg	47	27	37	31	142	8.5

3.4 Discussion

Plant Plus significantly reduced movements of *M. rufogriseus banksianus* through the linear corridor. Egg did not significantly reduce movements through the corridor when compared to the controls. The magnitude of the difference was not as striking as reported for the two-choice trials in Chapter 2. However, the mean number of movements through the Plant Plus barrier was approximately 20% fewer than the movements through the wet control barrier.

Plant Plus was most effective at reducing movements of *M. rufogriseus banksianus* immediately after application, during the afternoon period. The possible decrease in effectiveness following this time period may be an artefact of the procedure, an indication of rapid habituation, or an indication that the nature of Plant Plus rapidly changes after application. Alternatively, since no other food source was available, increasing hunger following initial aversion may have mitigated the effectiveness of Plant Plus. However, the reasons for, and the importance of the apparent change in effectiveness of Plant Plus requires further investigation.

There were many limitations in this trial. Subjects were pooled and while recovery periods were designed to keep samples independent, a violation of independence of samples occurred by using the same group of animals for each treatment and replicate. However, the method of data analysis was similar to those used for single subject mammalian bioassays (Nolte & Mason, 1998). The amount of replication of each treatment was also a limiting factor. The trial arena may have introduced error as non-target species were not excluded and may have influenced the behaviour of the test subjects. Additionally, the only permanent water source in the arena was relatively close to the treatment area and may have increased the necessity of the test subjects to encounter the treatments. While the results of this preliminary trial indicated that Plant Plus significantly reduced movements of *M. rufogriseus banksianus* it is important to note these limitations when interpreting the results.

Two-choice trials (utilised in Chapter 2) are usually sensitive in the detection of repellence, but one-choice (no-choice) trials are useful in testing the strength of repellents (see Nolte & Mason, 1998). The barrier trial was similar to a one-choice trial as subjects only had access to one food source, and to access it an encounter with the stimulus was necessary. One-choice trials often follow the occurrence of two-choice trials to assess the avoidance of stimulus without offering a confounding option (e.g. Nolte & Barnett, 2000). As such, the 20% reduction in movements by *M. rufogriseus banksianus* by Plant Plus in this trial is a promising result. As repellence is relative (see Nolte, 2003) and Plant Plus was temporarily effective when there was no alternative food source, Plant Plus should be at least as effective in situations where there are alternate food sources and habitats. It is expected that this would normally be the case in field situations.

The results of this barrier trial, when examined in conjunction with Chapter 2, highlight the potency of Plant Plus as an area repellent. As egg did not significantly reduce movements, it is recommended that further captive trials focus on Plant Plus, enabling adequate resources to stringently test Plant Plus. As the barrier trial has elucidated that Plant Plus can be used as an area repellent, the possible application methods for the reduction of macropod-vehicle collisions can be hypothesised and potentially tested.

The majority of research into products with area repellent properties has been discouraging, as the distance of effect is normally found to be short (e.g. ≤ 1 m) and/or the repellent effects are short-lived (Nolte, 2003). However, recently the odour associated with coyote hair has been revealed as an area repellent with more promising properties (Seamans *et al.*, 2002). Further investigation into the area repellent properties of Plant Plus, specifically assessing the distance of effect are necessary to determine if and how Plant Plus may be used in management situations.

If further investigation supports Plant Plus as an effective area repellent, Plant Plus could be used in the management of vehicle-macropod collisions with several possible modes of action. As an area repellent Plant Plus could potentially: reduce densities of macropods in road easements both directly and indirectly (reducing palatability of resources); or form a scent fence reducing the probability of vehicle-macropod collisions. As Plant Plus is sulfur-based and is likely to induce fear or an anti-predator response (see Section 2.4), Plant Plus may also be effective in increasing the vigilance of animals, which may also reduce vehicle-macropod collisions.

Road-based field trials have many inherent problems and can be financially unviable (Lintermans, 1997). As such, it is often more efficient to stringently test the underlying assumptions of roadkill mitigation strategies (Lintermans, 1997). Some of the underlying assumptions of Plant Plus as a roadkill mitigation strategy need further study. These include (but are not limited to):

- Stringent captive trials confirming results of screening and preliminary trials;
- The assessment of habituation to Plant Plus by macropods;
- Determination of the field life of Plant Plus;
- Establishment (and assessment) of a suitable application method and dosage for roadside use;
- Tests of repellence for multiple species (focusing on large macropods); and
- Impact on environment.

It is recommended that these assumptions are tested and that field trials proceed to confirm the results of captive trials with animals in their typical habitat.

Chapter 4

Habituation of *Macropus rufogriseus banksianus* to an odorous repellent

Chapter 4 Habituation of *Macropus rufogriseus banksianus* to an odorous repellent

4.1 Introduction

4.1.1 Background

Habituation is the decrease in response to stimuli following repeated exposures and is a process of the central nervous system (Thompson & Spencer, 1966). In a review of behavioural habituation, Thompson & Spencer (1966) identified nine characteristics of habituation. In summary these were:

1. Repeated applications of stimulus results in decreased response (habituation) and the decrease is often a negative exponential function of stimulus presentation;

2. Habituation will reverse over time in the absence of stimuli (spontaneous recovery);

3. If subjects are repeatedly habituated following spontaneous recovery, habituation becomes more rapid;

4. The rapidity of habituation is related to the frequency of stimulation;

5. The strength of stimulus is inversely related to rapidity of habituation;

6. Habituation can exceed the asymptotic response level, resulting in slower spontaneous recovery;

7. Habituation to stimulus can influence response to other stimuli (stimulus generalisation);

8. Presentation of another stimulus (strong) can result in recovery (dishabituation); and

9. Repeated application of dishabituating stimulus (point 8) can result in habituation of dishabituation response.

Thompson & Spencer (1966) demonstrated the above characteristics by investigating habituation to the spinal flexion reflex to electric shock in *Felis sp.* (cats). Their research also indicated that complex responses are more susceptible to habituation than simple responses.

Much of the recent work investigating habituation has been performed on different breeds of laboratory rats (*Rattus norvegicus*). Habituation to *Felis sp.* odour by *R. norvegicus* was apparent in laboratory trials investigating hiding behaviour. The use of anxiolytics and further testing in elevated mazes indicated that the response detected to *Felis sp.* odour was fear-based. The habituation occurred to a "modest level" of odour exposure (Dielenberg & McGregor, 1999).

Habituation was not detected over a five day trial period when investigating freezing behaviour in response to 2,4,5 trimethylthiazoline (TMT) by *R. norvegicus* (Wallace & Rosen, 2000). The subjects were exposed to the odour for 20 minutes on each day. Habituation was also absent within exposures as rats maintained the same level of anti-predator behaviour throughout the 20 minute trial periods. McGregor *et al.* (2002) reported that the response of *R. norvegicus* to TMT was not as strong as the response to *Felis sp.* odour and habituation was low to both odours.

Williams *et al.* (1990) investigated habituation and extinction of freezing in *R. norvegicus* in response to odours of cats and aggressive, alpha conspecifics. The fear of *Felis sp.* odours was not extinguished and is evident of a strong fear reaction. The response to conspecific odours was not as strong. It was postulated that habituation to *Felis sp.* odours would be slower than to odours of aggressive conspecifics (Williams *et al.*, 1990). Similarly, Zangrossi & File (1994) found little evidence of habituation by *Rattus norvegicus* to *Felis sp.* odour and that extinction of the response elicited by the odour was limited.

Blanchard *et al.* (1990) studied various stimuli (including *Felis sp.*) and the hiding response of *R. norvegicus* in a burrow system. Following presentation of *Felis sp.*, rats spent less time on top of the burrow system and this effect was apparent after repeated exposure. It was concluded that *Felis sp.* exposure was long lasting and responses were resistant to habituation.

The biological and neural aspects of habituation are largely unknown. File *et al.* (1993) investigated corticosterone concentrations in *R. norvegicus*, examining the link between avoidance behaviour, habituation and corticosterone concentrations in response to *Felis sp.* odour. While behavioural habituation to *Felis sp.* odour was not detected (see also Blanchard *et al.*, 1990; Williams *et al.*, 1990; Zangrossi & File, 1994; Wallace & Rosen, 2000), corticosterone concentrations did reduce following repeated exposures. It was concluded that there was dissociation between corticosterone concentrations and the behavioural response to *Felis sp.* odour (Note: habituation to cat odour has since been detected by Dielenberg & McGregor, 1999).

Yadon & Wilson (2005) reported that habituation to conspecific odours could be reduced by bilaterally infusing a glutamate receptor antagonist (cyclopropyl-4-phosphonophenylglycine) into the anterior piriform cortex in *R. norvegicus*. Similar results were reported by Best & Wilson (2004) and Best *et al.* (2005) suggesting that metabotropic glutamate receptors on cortical afferent pre-synaptic terminals play a significant role in short term habituation to odours. Several other neural processes are also implicated in habituation to odours (Best & Wilson, 2004; Yadon & Wilson, 2005).

Gilsdorf *et al.* (2003) reviewed the use of frightening devices in wildlife damage management. Focus was placed on visual and acoustic devices: however, habituation was reported as a major limiting factor in the utilisation of frightening devices. Some techniques to reduce or slow the rate of habituation were discussed and included: random or animal activated deployment of stimuli; the integration of several stimuli (creating a multifaceted repellent); repositioning of stimuli; and limiting the use of stimuli. Murray *et al.* (2006) also reported that providing resources to allow the avoidance of stimuli (e.g. untainted/alternative resources) reduced habituation.

The effectiveness of repellent odours is not independent of dose (see Takahashi *et al.*, 2005). Several studies have demonstrated that defensive, anti-predator and avoidance responses to odours are dose dependent (Gurney *et al.*, 1996; Wallace & Rosen, 2000; Takahashi *et al.*, 2005). Animals may also readily habituate to odour repellents (Beauchamp, 1995). Mason *et al.* (2001) identified habituation to fear inducing repellents as a major disadvantage for their use in wildlife management, and related the rate of habituation to the association between the fear-inducing odour and its perceived risk of predation. If the perceived risk of predation is low or has been removed, habituation is postulated to be rapid (Mason *et al.*, 2001). Similarly, McGregor *et al.* (2002) identified habituation to be more likely with specific non-reinforced predator cues rather than aversive stimuli, while File *et al.* (1993) found that habituation to disturbance occurs more readily than habituation to avoidance. It is speculated that rapid habituation of animals to odours (including repellents) during test procedures results in failure to detect responses that exist (Apfelbach *et al.*, 2005).

Epple *et al.* (2001; 2004) investigated the repellent qualities of the vapours (odour) of *Zanthoxylum piperitum* (Szechuan pepper). Investigations focussed on the feeding responses of *Microtus ochrogaster* (prairie voles) and *R. norvegicus*. No habituation by *R. norvegicus* was detected to vapours of *Z. piperitum* over five weeks involving biweekly (10) exposures (Epple *et al.*, 2001). Similarly, habituation was not detected in *M. ochrogaster* over 12 consecutive days of repeated exposure (Epple *et al.*, 2004).

Gurney *et al.* (1996) found that *Apodemus sylvaticus* (wood mice) rapidly habituated to cinnamamide (a synthetic plant-based repellent) but *Mus musculus* (house mice) did not habituate and showed a persistent aversion to the repellent. Gurney *et al.* (1996) concluded from these results that cinnamamide could be an effective repellent for *M. musculus*.

Arnould & Signoret (1993) assessed habituation in *Ovis aries* (sheep) to various repellent odours. Repellents were presented to subjects over seven to nine successive days. Sheep habituated (resumed feeding from odour tainted troughs) to odours of foetal fluids and Big Game Repellent (based on putrescent eggs), but did not habituate to dog faeces (Arnould & Signoret, 1993).

An investigation of feeding responses by *Microtys oeconomus* (root vole) in response to a predator odour found that *M. oeconomus* did not habituate to the scent of *Mustela erminea* (stoat) over 14 days in laboratory tests, even though the strength of the response was low (Borowski, 1998a). While habituation was not apparent, the odour (and synthetic components) were not recommended for use as a repellent due to inadequate strength of response.

Dog urine was effective in reducing *Wallabia bicolor* (swamp wallaby) damage to eucalypt seedlings over a six-week period. Although habituation to the urine was not the focus of the study, dog urine did appear to retain its effectiveness for the six week period, indicating habituation was minimal (Montague *et al.*, 1990).

Plant Plus has been the focus of a small number of investigations (Morgan & Woolhouse, 1995, 1998; Ramp *et al.*, 2005; Miller *et al.*, 2006). Morgan & Woolhouse (1995; 1998) investigated the use of Plant Plus (formerly known as Pine Plus and TOM) for reducing browsing damage by *Trichsorus vulpecula* (common brushtail possum) and *Oryctolagus cuniculus* (European rabbit) in New Zealand. Habituation was not directly investigated in either study, however Morgan & Woolhouse (1995) conducted a field trial lasting 81 days. Browsing by *T. vulpecula* on treated plants increased during the study period, however it was assumed that this was due to the repellent perishing (as it was not re-applied) rather than a habituation response forming in subjects through repeated exposure. Habituation was not investigated by Ramp *et al.* (2005), who investigated responses of *Macropus parma* and *Thylogale thetis* to Plant Plus or Miller *et al.* (2006) who conducted a multi-species field trial with Plant Plus.

Rapid habituation to repellents is a major limitation for their use in wildlife management as initial effectiveness can be quickly lost and not regained (Mason *et al.*, 2001; Apfelbach *et al.*, 2005). The efficacy of a repellent is reliant on its prolonged effectiveness in the field, which is determined by the habituation of target species to the repellent and the product-related longevity under ambient field conditions. Determination of the rate of habituation by a target species to a repellent is necessary to ensure effective management and is important when performing a cost-benefit analysis.

4.1.2 Aim

The aim of this trial was to investigate the response of *M. rufogriseus banksianus* to repeated exposures of Plant Plus. As habituation is a major disadvantage of repellents, the elucidation of habituation is important in the clarification of the repellent properties of Plant Plus. Specifically, the objectives of this trial were to:

- Further confirm the effectiveness of Plant Plus as a repellent for *M. rufogriseus banksianus* as indicated by previous trials (Chapters 2 & 3);
- Determine if the aversive response of *M. rufogriseus banksianus* to Plant Plus decreases over time (habituates); and
- Establish the rate of habituation in feeding response by *M. rufogriseus banksianus* to Plant Plus.

4.2 Methods

The trial described in this chapter received ethics approval from the Animal Care and Ethics Committee of the Director General of New South Wales Agriculture (Approval Number 02/1926 - 2) and also from the University of New South Wales (UNSW) Animal Care and Ethics Committee (Approval Number 03/68). The trial was designed to conform to the principles outlined in the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (NH&MRC, 2004) and relevant legislation that relates to the use of animals for scientific purposes in NSW.

This research was justified ethically and scientifically as this work is new, does not involve pain or discomfort to animals and was based on principles aimed at reducing wildlife mortality. Copies of the ethics approvals and National Parks and Wildlife Service permits are located in Appendix A.

4.2.1 Study Area

The habituation trial was conducted at the UNSW Cowan Field Station from November 2003 until June 2004. A description of the UNSW Cowan Field Station is provided in Section 2.2.1.

Enclosures B1 and B2 (Figure 2.1) were utilised for the habituation trial. Enclosures were adjoining and utilised as one large outdoor enclosure for the trial. The trial enclosure contained two self-filling water troughs, two feed sheds, additional artificial shelter and contained native and exotic grasses in addition to native sheltering trees. A similar enclosure at the field station was described as semi-natural (Hunt *et al.*, 1999) and is suitable for the maintenance of *M. rufogriseus banksianus* (Watson *et al.*, 1992).

The feed sheds in B1 (referred to as feed Tray A) and B2 (referred to as feed Tray B) were the only areas where pelleted feed (Doust and Babbage; Concord West) was available during the trial. The feeding sheds were separated by 20 m. Feed sheds and food trays were cleaned each day throughout the trial. Infrared cameras (1/3" CCD, black and white camera with 4.3 mm lens, and LEDs) were located in each feed shed. The cameras were used to monitor animals while approaching feed trays and feeding. The infrared cameras were connected to time-lapse videocassette recorders (TL VCRs, 9:1 real time: recorded time).

4.2.2 Study Subjects

Sixteen *M. rufogriseus banksianus* were involved in the habituation trial. Subjects belonged to a captive colony, but were not tame or habituated to human presence. The animals had not previously been used in odour related trials. Subjects were divided into four equal groups of four animals (Group 1 =3 females, 1 male; Group 2 =2 females, 2 males: Group 3 =3 females, 1 male: Group 4 =3 females, 1 male) and each group was trialled separately in the same enclosure. Each group of subjects were exposed to the same procedure. Animals were introduced to the trial enclosure and allowed to acclimate for seven days before the commencement of the trial. Animals had no previous exposure to odour repellents as part of any experiment. Animals had been previously used in an observational trial conducted by researchers from the UNSW. The previous trial had involved exposing the animals to flashing lights and recording behavioural responses.

4.2.3 Procedure

A captive, choice-based feeding format similar to the one described in Chapter 2 was utilised to assess habituation. Each day between 3:00 and 4:00pm (AEST), 1.5 kg of pelleted kangaroo food (Doust and Babbage, Concord West) was placed in each feed tray. Following the acclimation of animals to the trial enclosure, a pretrial assessment of food consumption was undertaken by weighing the amount of pelleted food in each of the two feed trays daily and analysing video surveillance for approach data. The method of data collection for consumption and approaches was the same as for Chapter 2 (Section 2.2.4). The pretrial period lasted for at least three days and continued until feeding patterns were stable.

The trial period began by placing 15 ml of Plant Plus (at recommended concentration) in a petri dish and attaching it to one of the feed trays (see Figure 2.2) using methods described in Section 2.2. After 24-hours, the repellent was removed, food at both feeding stations was weighed and replaced, and a new 15 ml sample of Plant Plus was dispatched. The feed station at which the Plant Plus was placed was reselected every 24-hours using a random number table. However, to avoid the potential for conditioned learning (see Garcia *et al.*, 1955), the placement of repellent at the same feed station for more than three consecutive days was never allowed. This regime was followed for each group of animals (non-concurrently) for a period of six weeks (Table 4.1). Feed areas were cleared and cleaned every 24-hours to remove faeces, urine and other contaminants from the area and feed trays were washed and replaced daily (3-4 pm AEST). Following the completion of the trials, all animals were returned to the care of UNSW Cowan Field Station staff.

Table 4.1 Details of the trial times for each group of subjects utilised in the habituation trial. * The length of the trial for Group 2 was reduced due to complications (see Results and Discussion for details).

	Commencement of pretrial period	**Completion of trial period**
Group 1 (3 females: 1 male)	12 November 2003	30 December 2003
Group 2 * (2 females: 2 males)	5 January 2004	30 January 2004
Group 3 (3 females: 1 male)	9 February 2004	27 March 2004
Group 4 (3 females: 1 male)	4 May 2004	21 June 2004

4.2.4 Data Analysis

The mass of food consumed from, and the daily number of approaches (head dips) by *M. rufogriseus banksianus* to each tray were the main dependent variables analysed for this trial. Time (trial day) was the independent variable. Data were screened for outliers, errors and normality.

Preference indices for both consumption and approaches were calculated following the methods outlined by Nolte & Mason (1998). This involved dividing the variable for the treatment tray by the total of the scores from each tray (e.g. If Plant Plus was located at Tray A than the preference score would be calculated as head dips to Tray A divided by the sum of head dips to Tray A and head dips to Trays B). Preference scores less than 0.5 indicate aversion to stimulus, while scores above 0.5 indicate preference to stimulus and 0.5 indicates no preference. One sample t-tests with a test value of 0.5 were used to assess if preferences existed in the pretrial consumption and approach data. The pretrial preference indices were calculated according to the example above with Tray A as the false treatment. As independence of pretrial data was not maintained alpha was set at 0.01.

The pelleted kangaroo feed (Doust and Babbage, Concord West) supplied for the first group of animals in the trials was a different size and shape to the pellets used for all other groups. The composition of the pellets was identical, but the pellets available to Group 1 were cylindrical in shape and much smaller. While the mass of food consumed per day by Group 1 subjects was comparable to Groups 2-4, consistently more head dips into feed trays were noted and it was presumed that this was due to pellet size and shape and the associated ease of handling and use by *M. rufogriseus banksianus*. To homogenise the data and make it comparable to other groups, the number of head dips to each tray for Group 1 was adjusted by dividing each head dip score by 2.75. This transformation coefficient was calculated by summing the total number of head dips per day of each trial and then dividing the total head dips for Group 1, by the average total head dips of Groups 2, 3 and 4.

Both parametric and non-parametric statistical analyses were performed (utilising SPSS 13.0 for Windows) to assess if feeding (consumption) or approaches to feed trays changed over time. Loess regression was utilised to model the trends in daily consumption and approach preferences. Both linear and exponential regression was performed with raw and preference data to assess trends in aversiveness over time. Linear regression was performed due to the robustness of the procedure and for detecting trends. Exponential regression was performed as habituation is often an exponential function (Thompson & Spencer, 1966; see Section 4.1 for review). Paired sample t-tests were used to assess differences between treatment and control trays.

To further assess the significance of any change in feeding preferences (transformed data) over time, raw data were collated for a week-by-week assessment by summing data for each tray into weeks (e.g. sum of days 1-7 per group formed the data subset for week 1: n=3). This allowed repeated measures analysis without pooling daily data, avoiding violations of independence similar to those termed as pseudoreplication by Hurlbert (1984). Due to the limited replication and heterogeneous nature of samples, non-parametric Friedman tests were used to analyse the data. When significant results were detected the mean ranks from the Friedman tests were used to speculate where differences occurred. Unfortunately, the low number of paired samples (replication) restricted the use of Wilcoxon Signed Rank tests to detect these differences.

4.3 Results

Unexpected within trial variance was detected for Group 2 during initial data screening. The unexpected variance appeared in both consumption and approach data but was more pronounced in the approach data. Variance for total approaches (head dips to treated tray + head dips to non-treated tray) for Group 2 was greater than the variance for the other groups by a magnitude in the order of 10. To further check for errors, the approach data for Group 2 were overlayed with the average approach data for Groups 1, 3 and 4 (Figure 4.1). A similar figure was created for the consumption data (Figure 4.2). From the overlays, it was quite clear that the responses observed for feeding and approaches for Group 2 were different than the responses for Groups 1, 3 and 4. The preference indices for Group 2 also had large variance with the range in approach preference (0.65) double the range for any other group (Group 1= 0.31, Group 3=0.29, Group 4=0.33). Due to this extremely high variance and dissimilarity to other replicates (groups), the Group 2 data set was excluded from further analysis.

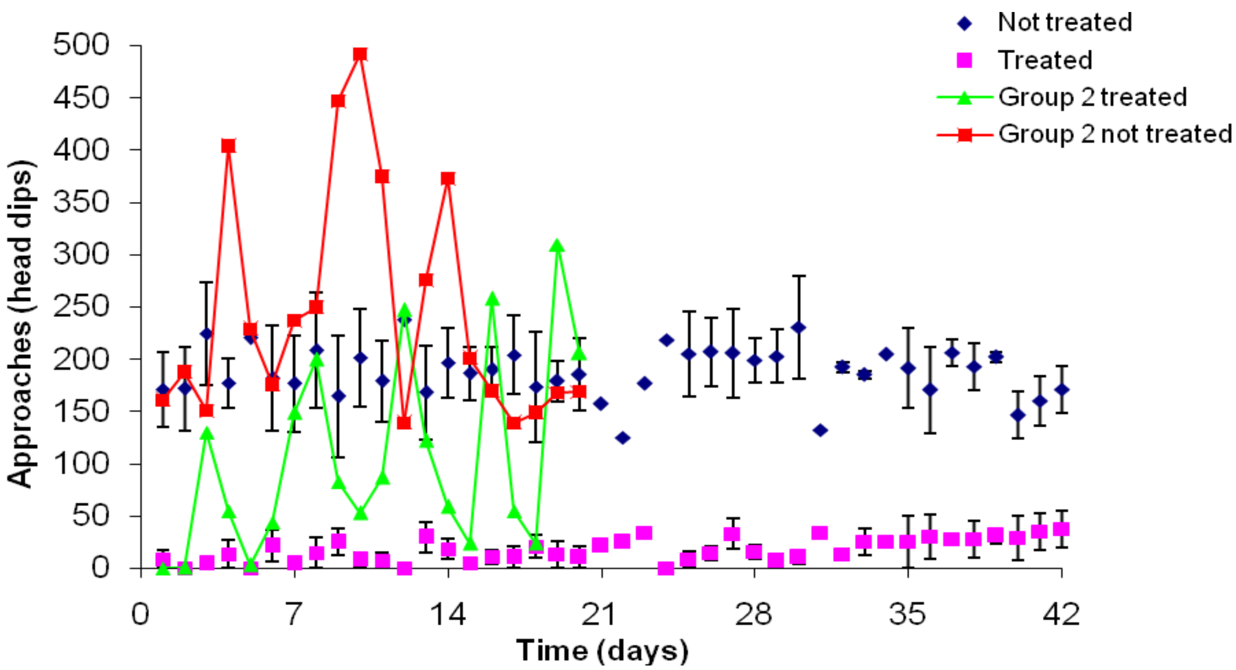

Figure 4.1 Mean number of approaches (head dips) to the treated and non-treated feed trays for Groups 1, 3 and 4. The raw data for Group 2 subjects were overlayed. Error bars indicate one standard error. Note: Error bars are absent for days 5, 12, 21, 22, 23, 24, 31 and 34 as n<3.

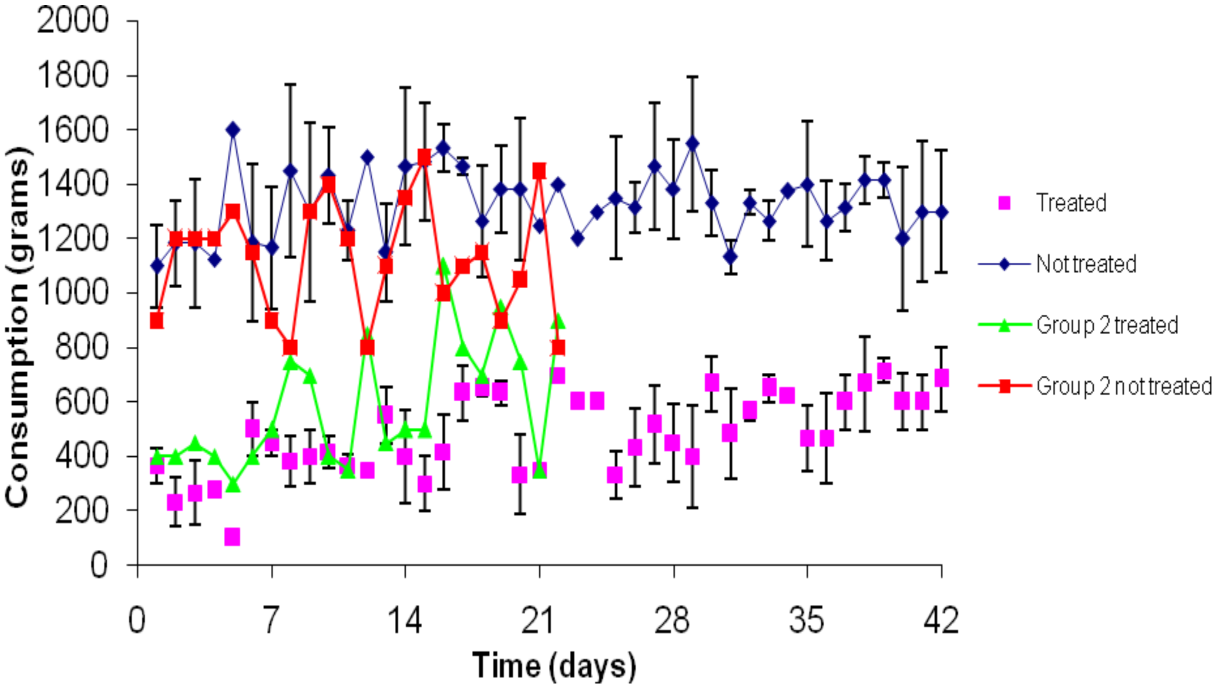

Figure 4.2 Mean consumption of pelleted food (g) from the treated and non-treated feed trays for Groups 1, 3 and 4. The raw data for Group 2 subjects were overlayed. Error bars indicate one standard error. Note: Error bars are absent for days 5, 6, 12, 21, 22, 23, 24, and 34 as n<3.

Pretrial preferences in consumption and approaches to feed trays were assessed overall and group by group with two-tailed, one-sample t-tests (Table 4.2). A slight but significant preference toward Tray A was detected in consumption values for Group 3. The magnitude of the preference was very small and no significant preference was detected towards either tray in approaches for Group 3 (Table 4.2). No preference was detected in approaches of consumption for Groups 1 or 4 and similarly no significant preferences were detected overall.

Table 4.2 Pretrial preferences in approaches and consumption. Values greater that 0.5 indicate a preference to Tray A, values less than 0.5 indicate preference to Tray B and 0.5 is indicative of no preference. Significant results are highlighted. n=3 for Group 1, n =5 for Groups 3 and 4. Overall preference was calculated using the average pre trial data from each group as a replicate (n=3).

	Approach preference			**Consumption preference**		
	mean	t score	p value	mean	t score	p value
Group 1	0.45	-1.84	>0.01	0.39	-1.39	>0.01
Group 3	0.53	0.88	>0.01	0.56	12.5	<0.01
Group 4	0.35	-4.41	>0.01	0.41	-2.75	>0.01
Overall	0.44	-1.09	>0.01	0.45	-0.87	>0.01

A scatter plot of consumption preference per day with loess regression line indicates that a small increase in consumption preference (or decrease in aversiveness of treated tray) may have occurred over the 42 days of the habituation trials (Figure 4.3). A linear regression indicated that there was a modest, significant increase in consumption preference over time (r^2=0.11, F[1,113]=13.6, p<0.0005). A similar relationship was found between the raw consumption data from treated tray and time (r^2=0.20, F[1,113]=28.02, p<0.0005). No similar or inverse relationship was detected between the consumption of pelleted food from the untreated tray and time (r^2<0.01, F[1,113]=0.484, p>0.05).

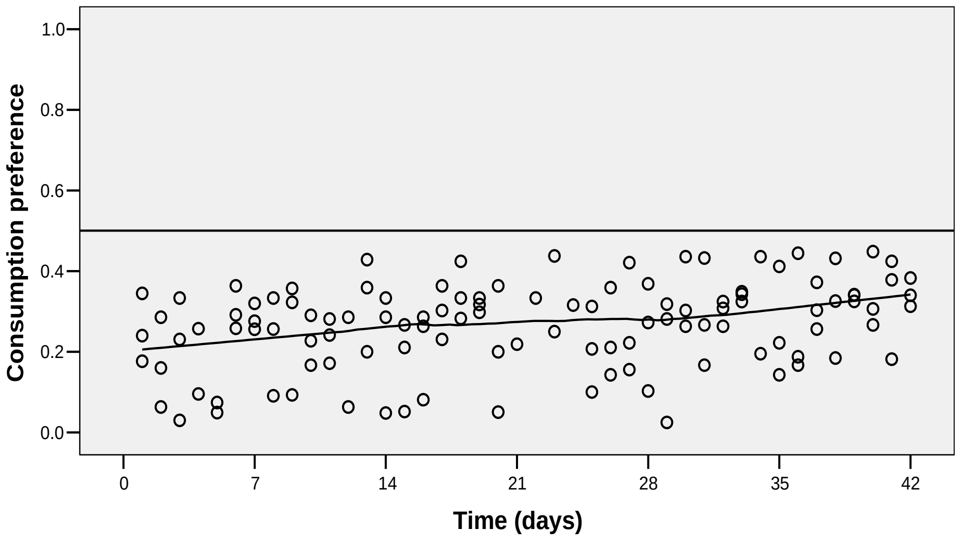

Figure 4.3 Scatter plot of consumption preference with loess line of fit. Values less than 0.5 indicate aversion to treated tray. Values greater than 0.5 indicate preference to treated tray. A value of 0.5 indicates no preference (reference line). A linear relationship was evident (r^2=0.11, F[1,113]=13.6, p<0.0005).

Similarly, a scatter plot of approach preference with loess regression line (Figure 4.4) indicated an increase in approach preference (decrease in aversiveness of treatment) over the 42-day period. An exponential regression of approach preference (transformed to head dips + 1, due to the occurrence of zeros in the data set) and time indicated that there was a small, significant increase in approach preference over time ($y=1.04e^{0.0024x}$, r^2=0.09, F[1,113]=11.34, p<0.001). This trend was also apparent in the raw data for approaches to the treatment tray

(r^2=0.16, $F[1,114]=21.86$, $p<0.0005$). No similar or inverse relationship was detected between approaches to the untreated tray and time ($r^2<0.01$, $F[1,113]=0.02$, $p>0.05$)

It is important to note that no preference values obtained for either variable (consumption preference, Figure 4.3; or approach preference, Figure 4.4) were 0.5 or above. All values were less than 0.5 indicating aversion from the treated trays throughout the trial for both indices.

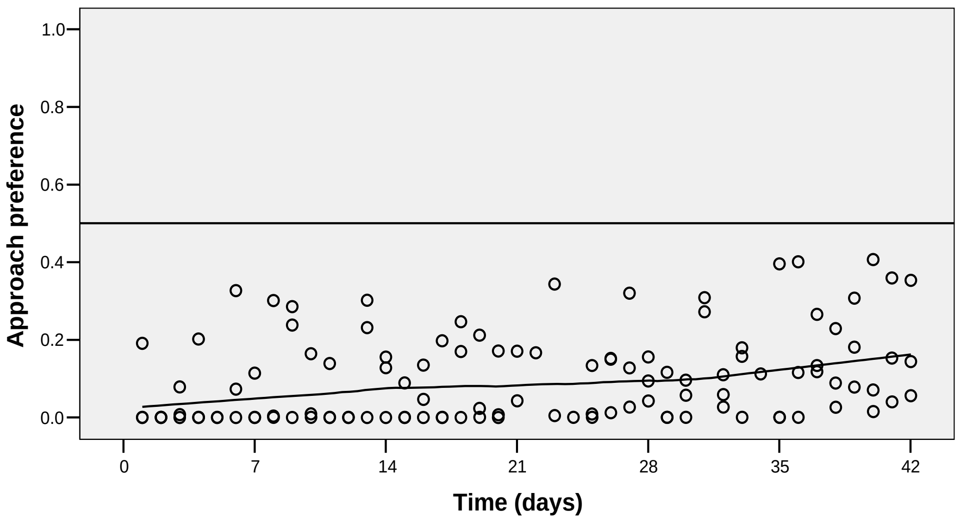

Figure 4.4 Scatter plot of approach preference with loess line of fit. Values less than 0.5 indicate aversion to treated tray. Values greater than 0.5 indicate preference to treated tray. A value of 0.5 indicates no preference (reference line). An exponential relationship between approach preference and time was evident ($y=1.04e^{0.0024x}$, r^2=0.09, $F[1,113]=11.34$, $p<0.001$).

Friedman tests to assess the significance of changes over time in consumption at a weekly scale were performed and no significant changes in consumption were detected between weeks for either the treated tray (χ^2_5=7.89, n=3, $p>0.05$) or untreated tray (χ^2_5=8.69, n=3, $p>0.05$). However, a non-significant increase in consumption at the treated tray was apparent over the six-week period (week 1 $\underline{M}$=2283 g - week 6 $\underline{M}$=4258 g: Figure 4.5). Paired sample t-tests revealed that at every time interval, significantly less pelleted food was consumed at treated trays, than at untreated trays (Figure 4.5).

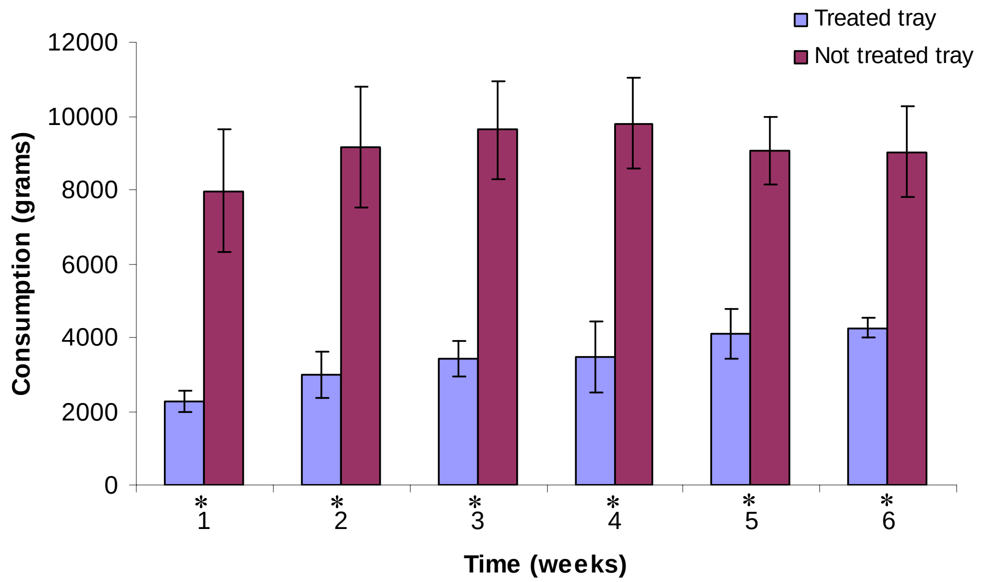

Figure 4.5 Mean weekly consumption of pelleted food (g) from trays treated with Plant Plus and trays without repellent (±1 std error). Friedman analyses did not detect significant differences within treated tray samples (between weeks: χ^2_5=7.89, n=3, p>0.05) or non-treated tray samples (χ^2_5=8.69, n=3, p>0.05). * indicates a significant difference (p<0.05) between treated and untreated trays (one-tailed paired samples t-tests).

The number of approaches made to treated feed stations by *M. rufogriseus banksianus* did change with time (Friedman test: χ^2_5=11.95, n=3, p<0.05), with the lowest weekly mean of 80 approaches in Week 1 and the highest weekly mean of 244 approaches in Week 6 (Figure 4.6). The mean ranks utilised by the Friedman test for the analysis for weeks four, five and six were the highest (4.3, 4.7 and 5.3 respectively), while week one had the lowest mean rank (1.0). The mean ranks for weeks two and three were intermediary (3.7 and 2.0 respectively).

The number of approaches by *M. rufogriseus banksianus* to non-treated feed stations did not differ significantly between weeks (χ^2_5=1.10, n=3, p>0.05) with a small range of means (1186-1381). Paired sample t-tests between the number of approaches made to treated trays and untreated trays each week, revealed that significantly fewer approaches were made to treated trays for each week with the exception of week five (Figure 4.6). The result for week five was nearing significance (t=2.69, n=3, p=0.058) and the lack of significance is most likely an artefact of high variance and inadequate replication.

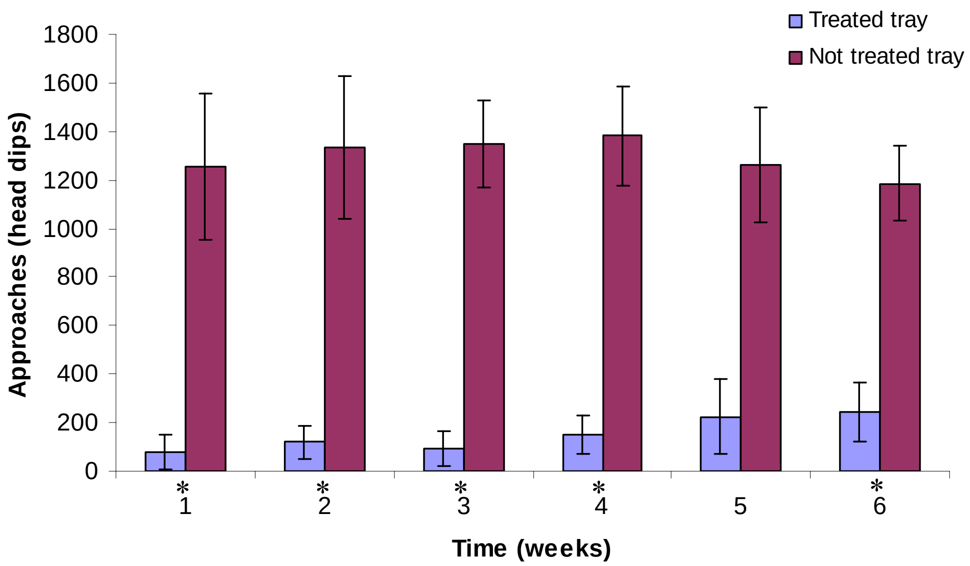

Figure 4.6 Mean weekly approaches (head dips) to trays treated with Plant Plus and trays without repellent (±1 std error). A Friedman analysis detected a significant difference within treated tray samples (between weeks: χ^2_5=11.95, n=3, p<0.05). No significant difference was detected within untreated tray samples (χ^2_5=1.10, n=3, p>0.05). * indicates a significant difference (p<0.05) between treated and untreated trays (one-tailed paired samples t-tests). Note: The one-tailed paired samples t-test to compare approaches to treated and untreated trays during week 5 returned a result close to significance (t=2.69, n=3, p=0.057).

4.4 Discussion

The effectiveness of Plant Plus in reducing feeding and approaches to feed trays by *M. rufogriseus banksianus* was confirmed by these habituation trials. Positive trends indicating habituation were detected between both consumption and time, and approaches and time. However, trends were not strong and Plant Plus was still aversive to *M. rufogriseus banksianus* at the end of the trials (Figures 4.3 and 4.4). A significant increase in the weekly number of approaches to feeding trays where Plant Plus was present was detected over the six-week trial period (Figure 4.6), also indicating some habituation. A similar but non-significant tendency was detected in the weekly mass of food consumed from treated trays (Figure 4.5).

The lack of significance in the increase of mass of food consumed per week may be due to increased variance stemming from the access of non-captive *Trichosurus vulpecula*, *Rattus norvegicus* and a variety of birds to the trial arena. The presence of *R. norvegicus* and the birds was difficult to quantify: however, they were noted to attend all feed stations. The presence of *T. vulpecula* at each feed station was quantified through analysis of surveillance footage and was noted with equal frequency at each feed station. While potentially confounded by the presence of the other species, the consumption index was positively correlated with the approach index (r=0.79) further indicating that the effect of the confound may be small. However, the approach data, collected from the analysis of time-lapse video, removes the confounding effects of the pest species and may be more reliable.

The rate of habituation to Plant Plus by *M. rufogriseus banksianus* was not detected with confidence in these trials as habituation was slow and some variation in response was detected. The trends detected were significant but not strong and Plant Plus remained effective over the six-week period. Habituation is often a negative exponential of stimulus presentation (Thompson & Spencer, 1966). In this trial, time was relative to stimulus presentation. The number of approaches to the treated tray as well as approach preference followed exponential trends with time. Extrapolation of the regression equation for approach preference and time ($y=1.04e^{0.0024x}$) predicts habituation to Plant Plus (indicated by a preference score of 0.5) would be complete approximately 21 weeks into the repeated exposure regime. However, due to the size of extrapolation (greater than three times the length of the trial) and variance of results, this figure must be treated with extreme caution.

Despite being a major disadvantage of repellents, habituation by target species to odorous repellents is not often established (Mason *et al.*, 2001). Arnould & Signoret (1993) detected habituation to both conspecific foetal fluids and MGK Big Game Repellent when investigating feeding preferences in *O. aries*. However, habituation to canine faeces was not detected over nine days and resulted in further trials to assess the value of canine urine and synthetic predator odours as repellents for ungulates (Arnould & Signoret, 1993; Arnould *et al.*, 1998).

Gurney *et al.* (1996) assessed habituation of feeding responses by *Mus musculus* and *Apodemus sylvaticus* to cinnamamide over three days. *Apodemus sylvaticus* habituated over the trial period, but habituation was not detected for *M. musculus* and it was suggested that cinnamamide had potential as a repellent for this species. Further trials with *Mus musculus* and *Rattus norvegicus* supported the use of cinnamamide as a mammal repellent (Watkins *et al.*, 1998; Gill *et al.*, 2000). However a recent trial with *Meles meles* (European badger) yielded less encouraging results (Baker *et al.*, 2005).

Following several trials, including the assessment of habituation, Epple *et al.* (2001; 2004) suggested that *Zanthoxylum piperitum* may be useful as a feeding deterrent in an integrated management strategy for reducing damage caused by *Microtus ochrogaster* (prairie voles).

The habituation trial with *M. ochrogaster* ran for 12 consecutive days: habituation was minimal (Epple *et al.*, 2004).

The response to Plant Plus by *M. rufogriseus banksianus* was consistent across three groups of animals involved in this trial indicating habituation at low levels over the six-week period. However, the response to Plant Plus by subjects of Group 2 highlighted the variation in response. There are many possible reasons for the different response observed for Group 2, and could include social facilitation, gender discrepancies and methodological error.

While generally regarded as solitary animals, *M. rufogriseus banksianus* do socially interact and have a social organisation similar to other gregarious macropods (Johnson, 1989a). As such, sociality of animals may have influenced feeding behaviour and responses to Plant Plus. Social facilitation (e.g. where the interaction of subjects influences behaviour) and its effects on feeding behaviours in response to a repellent was investigated in *Ovis aries* by Arnould & Signoret (1993) using anosmic and intact subjects. Social facilitation did not influence the repellency of canine faeces for *O. aries* as intact ewes still avoided food tainted with odours even in the company of anosmic subjects which fed from tainted trays (Arnould & Signoret, 1993). While the effects of social facilitation can not be excluded from these trials without specific investigation, the results obtained with *O. aries* by Arnould & Signoret (1993) indicate it may be unlikely to be a significant factor with *M. rufogriseus banksianus*. The reasons why any potential social facilitation would have affected Group 2 differently than Groups 1, 3 and 4 would also need further investigation.

There were two male and two female *M. rufogriseus banksianus* in Group 2, while Groups 1, 3 and 4 were comprised of three females and one male. Many gender related issues could have been involved in the discrepancy of the response observed. While breeding occurs throughout the year, there is a peak in birth rates of *M. rufogriseus banksianus* in the summer months (Calaby, 1983). Females come into oestrous shortly after birth and females in close association (e.g. overlapping home ranges or in captivity) often reach oestrous in synchrony (Johnson, 1989b; Watson *et al.*, 1992). It is possible that during the trial period for Group 2, both females could have been in oestrous, which could have affected feeding behaviour directly or indirectly (e.g. social interactions around the limited number of feed stations). Additionally, it is likely that the males may have been interacting in a dominant/sub-ordinate relationship or competing for these positions (Johnson, 1989b; Watson *et al.*, 1992), potentially affecting feeding, and responses to Plant Plus. It has also been noted that male *M. rufogriseus banksianus* spend less time feeding than females (Coulson, 1999), which may contribute to the variance observed.

Methodological constraints may also have led to spurious results from Group 2. The Plant Plus used for Group 2 was from the same batch used for Group 1 subjects, and while stored according to the manufacturers instructions, it may have been possible that the Plant Plus spoiled. Additionally, the trial arena may have become contaminated by faeces, urine or through spillage of Plant Plus, however this was not observed. The variation in response could also represent a natural variation in response of *M. rufogriseus banksianus* to Plant Plus. The reasons for the variations observed with Group 2 cannot be confidently determined. However, the variation in responses noted should be considered in future investigations, and also in the use of Plant Plus as a repellent or management tool.

Thompson & Spencer (1966) related the frequency of stimulus presentation to the rapidity of habituation. In these trials, Plant Plus was constantly in the trial arena and encounters with the stimulus were frequent. However, habituation was still only detected at low levels. If Plant Plus were to be deployed as a repellent in a situation where encounters between *M. rufogriseus banksianus* and the repellent were fewer, habituation would be expected to be even more gradual.

The strength of stimuli has been inversely related to habituation (habituation is rapid to weak odours) and response to aversive stimuli is dose dependant (Thompson & Spencer, 1966; Wallace & Rosen, 2000; Takahashi *et al.*, 2005). The application method of Plant Plus in this trial, followed the same presentation method as for Chapter 2, and was based on advice from the manufacturer. The application method of 15ml of Plant Plus in a petri dish on the food tray was determined as a low dose. Assuming habituation to Plant Plus by *M. rufogriseus banksianus* followed a dose dependant relationship (see Thompson & Spencer, 1966 and Takahashi *et al.*, 2005), habituation would be more rapid if application rates were reduced. When considering future trials or the use of Plant Plus as a repellent, it should be noted that reduced concentrations or application rates of Plant Plus may increase the rate of habituation. However, increasing concentrations and application rates could reduce habituation further. Plant Plus is available from the manufacturer at twice the recommended concentration for the purposes of cheaper distribution and transport. The concentrate was diluted with water to the recommended strength for use in these trials. Utilising a range of dilutions in a captive trial may enable elucidation of the dose/response relationship and provide further evidence of habituation, which may be beneficial if wide scale use of Plant Plus was to be considered.

Ramp *et al.* (2005) described the responses of *Thylogale thetis* and *M. parma* to Plant Plus as an anti-predator strategy. The response of *T. vulpecula* and *O. cuniculus* to Plant Plus (as Pine Plus and TOM) were assumed to be anti-predator in nature (Morgan & Woolhouse, 1995, 1998) as Plant Plus is based on the chemistry of canine urine. McGregor *et al.* (2002) differentiated the behavioural responses of *Rattus norvegicus* to *Felis sp.* odour from those to an extract of fox faeces (2,4,5 trimethylthiazoline: TMT) and reported that *Felis sp.* odour elicited defensive responses and was a predator cue. However, TMT did not elicit anti-predator or defensive behaviour, but was aversive, possibly due to its noxious qualities. Furthermore, McGregor *et al.* (2002) postulated that habituation to predator cues or odours would be more rapid than habituation to aversive stimuli.

The behavioural response of *M. rufogriseus banksianus* to Plant Plus may be related to anti-predator strategies, but investigations further describing behavioural responses may be useful in determining why Plant Plus is repellent. Further elucidation of behavioural responses of *M. rufogriseus banksianus* in response to Plant Plus would help develop Plant Plus as a wildlife mitigative strategy by highlighting its potential strengths, weaknesses and uses.

The response quantified during this trial (feeding) was simple and response was easily detected. Thompson & Spencer (1966) reported that habituation of complex responses is more probable than habituation of simple responses. While Plant Plus may have elicited other undetected responses from *M. rufogriseus banksianus*, the observed response was simple and may have contributed to the minimal levels of habituation detected.

The use of Plant Plus in an integrated management plan for *M. rufogriseus banksianus* may also reduce habituation. The use of several application and deployment methods of Plant Plus, aimed at reducing frequency of contact by animals with stimulus and increasing the dose of stimulus may further reduce habituation by animals in field situations (see Gilsdorf *et al.*, 2003).

As Plant Plus has been successful in reducing food consumption and related behaviour, and habituation was noted at only low levels following six weeks, it is recommended that further trials to assess Plant Plus as a repellent for the mitigation of vehicle-macropod collisions proceed.

Chapter 5

Longevity of an odorous repellent for *Macropus rufogriseus banksianus*

Chapter 5 Longevity of an odorous repellent for *Macropus rufogriseus banksianus*

5.1 Introduction

5.1.1 Background

The longevity of a repellent is an important factor that determines the viability of using a particular repellent in a management situation by influencing: application frequency; overall effect size; and cost effectiveness. The longevity of a repellent refers to the prolonged effectiveness of a repellent (see Swihart *et al.*, 1991) and is determined by two main factors: product-related longevity; and habituation to the repellent by the target species (see Chapter 4). The evaporative loss of volatile components and the denaturing of active ingredients are two major components that determine the product-related longevity of a repellent. It is necessary to separate the effects of product-related longevity from habituation in order to establish suitable application regimes for effective management plans: if a product's longevity is primarily determined by habituation, it is unlikely that reapplication of the repellent will be effective in restoring any observed reduction in a repellent's effect. Apfelbach *et al.* (2005) postulated that some potentially viable repellents may have been disregarded during preliminary screening if longevity (product-related or habituation) of the repellent was short.

Longevity is usually tested during field trials or long-term, browsing-based, captive trials (Montague *et al.*, 1990; Morgan & Woolhouse, 1995; Rosell & Czech, 2000; Santilli *et al.*, 2004; Baker *et al.*, 2005). However, in such trials, the reasons for the declining effect of repellents (habituation or product related) cannot be determined and many confounding factors are present (prevailing weather conditions, availability of resources, densities of target species). To overcome these issues, Swihart *et al.* (1991) elucidated the product related longevity of bobcat and coyote urine by assessing the effectiveness of the repellents under different application frequencies. The effectiveness of the urines remained high with frequent re-application and declined with an increase in period between applications. This indicated that product-related components (not habituation) were primarily responsible for the longevity of the repellent.

Plant Plus is an effective short-term repellent (Chapters 2, 3 & 4). There is evidence that *Macropus rufogriseus banksianus* do not habituate rapidly to Plant Plus (Chapter 4), however, the product-related longevity of Plant Plus has not been determined.

5.1.2 Aims

The aim of this trial was to investigate the length of time Plant Plus would remain an effective repellent for *M. rufogriseus banksianus* when exposed to ambient environmental conditions. The length of time repellents can remain viable in the environment is important when considering uses of repellents and application methods. Clarification of the product-related longevity of Plant Plus under ambient conditions is important for the use of Plant Plus *in-situ*. Specifically, the objective of this trial was to:

- Assess the product-related longevity of Plant Plus following extended exposure to environmental conditions on captive *M. rufogriseus banksianus*.

5.2 Methods

The trial described in this chapter received ethics approval from the Animal Care and Ethics Committee of the Director General of New South Wales Agriculture (Approval Number 02/1926-2) and also from the University of New South Wales (UNSW) Animal Care and Ethics Committee (Approval Number 03/68). Copies of the ethics approvals and the National Parks and Wildlife Service Permit are located in Appendix A.

5.2.1 Study Area

This longevity trial was conducted in May and June 2004 at the UNSW Cowan Field Station. A description of the field station is provided in Section 2.2.1.

Enclosure B3 (Figure 2.1) was utilised for the trials. Two temporary feeding shelters (TeamPoly™ Calf Shelter 1.93 m x 1.25 m x 1.25 m) were placed in the enclosure and used as feeding shelters throughout the trial. A permanent feed shed was also located in the enclosure but was only used for shelter during the trial. The enclosure contained native vegetation, however, due to prevailing dry weather conditions, ground cover was minimal. The enclosure was suitable for the maintenance of *M. rufogriseus banksianus* (Watson *et al.*, 1992).

Pelleted kangaroo feed (Doust and Babbage; Concord West) was provided in feed trays in each of the temporary feeding shelters. The enclosure contained a self-filling water source that animals had free access to at all times. The water source was checked and cleaned regularly throughout the trial.

5.2.2 Study Subjects

Eleven *M. rufogriseus banksianus* (7 female: 4 male) were involved in the trials. Animals belonged to a captive colony, but were not tame or habituated to human presence. Animals were not separated and remained as a group throughout the trial. Animals had no previous exposure to odour repellents as part of any experiment. Animals had been previously used in an observational trial conducted by researchers from the UNSW. The previous trial had involved exposing the animals to flashing lights and their recording behavioural responses.

5.2.3 Procedure

A two-choice feeding format, similar to the trials described in Chapters 2 and 4, was utilised to assess four different Plant Plus treatments. The treatments consisted of 15 ml of Plant Plus (recommended concentration) in a petri dish aged to four different periods (Table 5.1). The treatments were prepared before the commencement of the trial and were left in a semi-sheltered area (open-sided shed). This allowed exposure to ambient conditions but avoided exposure to rain. The distance between the field station and the area where the treatments were left to age was greater than 10 kilometres, thus avoiding premature exposure to study animals.

Table 5.1 Age of Plant Plus for treatments used in the longevity trial

	Length of exposure to conditions
Treatment A	1 week
Treatment B	10 weeks
Treatment C	22 weeks
Treatment D	32 weeks

Following the seven-day period of acclimation for subjects to the study area, a pretrial period ensued and the consumption of food from each feed station was monitored. The consumption of food and the number of approaches by *M. rufogriseus banksianus* was monitored daily following the methods described in Section 2.2.4.

The trial period consisted of four, 24-hour tests for each of the four treatments. A recovery period of at least 24-hours preceded each test. The order of tests within the trial period was random. During each day of the trial, between 3:00 and 4:00 pm AEST, pelleted food was placed in each feeding tray. On each test day, a treatment was collected from the aging area and brought to the field station. The treatment was attached to a randomly selected feed station using the methods described in Section 2.2.4. An empty petri dish was attached to the alternative feed tray.

Consumption of pelleted food and approaches to the feed tray were calculated each day using the same methods described in Chapter 2 (Section 2.2.4). Observations of animal behaviour were made from video surveillance where possible. Following the completion of the two-choice feeding trials, animals were returned to the care of the UNSW Cowan Field Station staff and carefully monitored.

5.2.4 Data Analysis

The mass of food consumed from each tray, and the number of approaches (head dips by *M. rufogriseus banksianus*) to each tray, were the main dependent variables analysed for this trial. Treatment (Table 5.1) was the independent variable. Similarly to Chapter 2, the recovery periods between each trial were designed to retain independence between tests. However, as each test was performed on the same set of subjects, a violation of independence occurred.

Preference indices were calculated for both mass of food consumed (consumption) and the number of approaches following the methods described by Nolte & Mason (1998). Preference indices were calculated by dividing the value for the treated tray by the sum of the treated and control tray values. Preference values less than 0.5 indicate a preference for the control tray, values more than 0.5 indicate a preference to the treated tray with 0.5 indicating no preference (equal values). Pre-trial preferences were calculated as value at Tray A divided by the sum of value at Tray A and Tray B.

The significance of preferences were calculated using one-sample t-tests comparing the consumption preference and approach preference to 0.5. One tailed, paired sample t-tests were utilised to assess the effect of each treatment against its paired control. Some results were superficially compared to transformed data collected on Day 1 of the habituation trial (Chapter 4). These data sets were obtained using similar methods and limited comparisons can be made with caution. However, direct statistical comparisons were not performed due to small sample sizes and slight differences in methods.

While several one-tailed, paired samples t-tests were performed, the Bonferroni correction was not applied as it increases the likelihood of Type II errors and has mathematical, logical and practical limitations (Moran, 2003). Bernoulli equations to test the likelihood of returning multiple significant results were not utilised, as consumption and number of approaches were not independent (an assumption of the Bernoulli equation). Due to the available levels of replication, alpha levels were not reduced, but caution was exercised in interpretation of results. Statistical analyses were performed with SPSS 13.0 for Windows (SPSS Inc, 2004).

5.3 Results

Pre-trial preferences in consumption of food and the number of approaches to feed trays by *M. rufogriseus banksianus* were assessed with two-tailed, one-sample t-tests. No pre-trial preference for either tray was detected for both consumption ($F=0.8$, $p>0.05$) and the number of approaches ($F=5.5$, $p>0.05$).

The consumption preference for each of the treatments is displayed in Figure 5.1. The 1-week and 10-weeks treatments had means that were significantly less than 0.5 ($F=62.3$, $p<0.05$ and $F=8.5$, $p\leq0.5$ respectively) indicating preference in feeding to the control tray. No significant departure from 0.5 (no preference) was detected for either the 22-weeks and 32-weeks treatments. A summary of the consumption data is tabled in Appendix E.

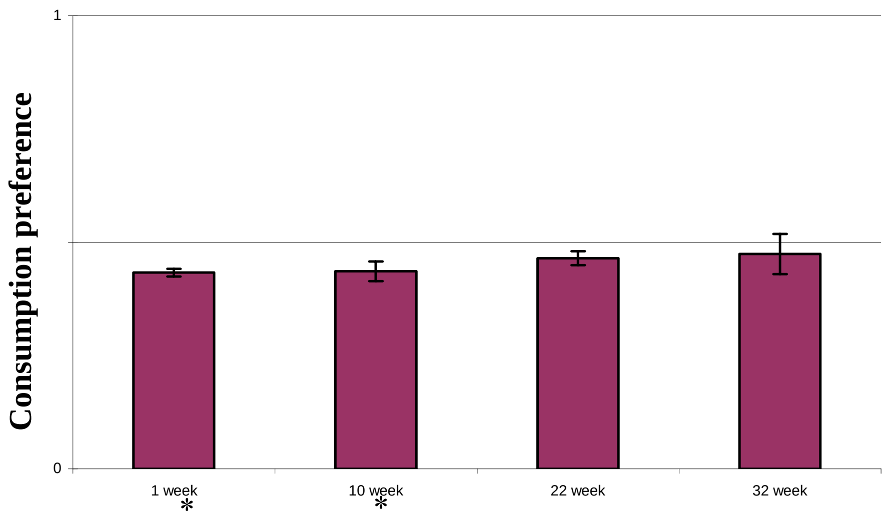

Figure 5.1 Consumption preference (mass of food consumed from treated tray divided by total mass of food consumed). Values less than 0.5 indicate less food consumed from treatment tray than from control tray. Values greater than 0.5 indicate more food consumed at treatment tray than control tray. Error bars represent 1 standard error. Note: * indicates a significant preference ($p\leq0.05$).

The approach preference (calculated from the video surveillance analysis) was lowest for the 10-weeks treatment (Figure 5.2) and was the only treatment with a mean significantly lower than 0.5 ($F=9.3$, $p<0.05$) indicating a preference for *M. rufogriseus banksianus* to feed from the control tray. It was noted during the video surveillance analysis that on 12 June 2004 the treatment feed tray was knocked over inside the feeding shelter by one subject. The treatment was seen in the video to contaminate the shelter and soil. On the following day, the area was raked clean, however the contamination on the feed shed was not noted or cleaned. The treatment that was in use on 12 June 2004 had been aged for one week.

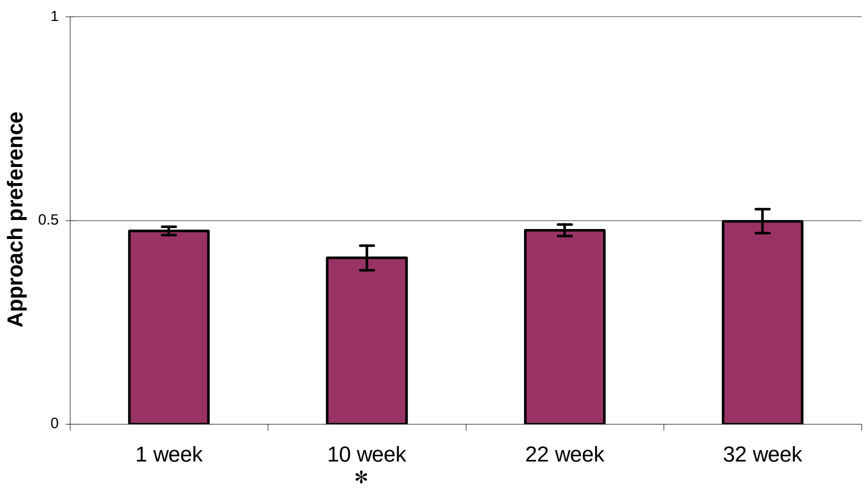

Figure 5.2 Approach preferences. Values less than 0.5 indicate less food consumed from treatment tray than from control tray. Values greater than 0.5 indicate more food consumed at treatment tray than control tray. Error bars represent 1 standard error. Note: * indicates a significant preference ($p\leq0.05$).

Due to contamination and the failure to note and rectify the contamination, all data collected after 12 June 2004 should be excluded as contamination of one feed shed continued and potentially affected subsequent trials. The 10-weeks treatment group had already been repeated three times by 12 June 2004 but was the only group to have done so. Due to the potential difference in trial conditions, data for the 1-week, 22-weeks and 32-weeks treatment groups will be excluded from further analysis. However, a summary of all approach data for the longevity trial is tabled in Appendix E.

The mean mass of food consumed from the treated tray (1783 g) was significantly less than the mean mass of food consumed from the control tray (2283 g) for the 10-weeks treatment ($F=10.7$, $p<0.05$). A similar outcome was noted in the results of the habituation trial (Chapter 4) for Plant Plus aged for one day (Figure 5.3). An analysis to determine the interaction effect and effect size between the 10-weeks treatment and day one of the habituation trial was not performed due to the slight differences in methods between the habituation trial and longevity trial and because of low sample sizes.

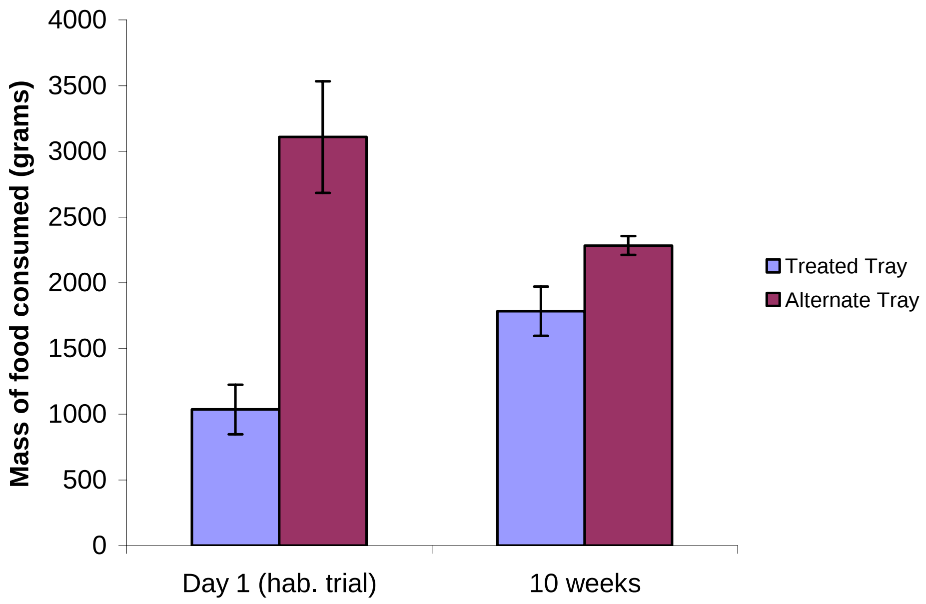

Figure 5.3 Mass of food consumed at treated and control trays for the 10-weeks treatment and for Day 1 of the habituation trials (Chapter 4). Note: error bars represent 1 standard error.

The mean number of approaches to the treated tray (625) was significantly fewer than the mean number of approaches to the control tray (908) for the 10-weeks treatment. ($F=8.8$, $p<0.05$). A similar outcome was noted during the habituation trials (Chapter 4) for samples from day one (Figure 5.4). Observational notes taken during video analysis indicate that treatments were approached and licked by subject/s during the 32-weeks treatment (all replicates) and 22-weeks treatment (two of three replicates). No licking was noted during any of the 1-week or 10-weeks trials.

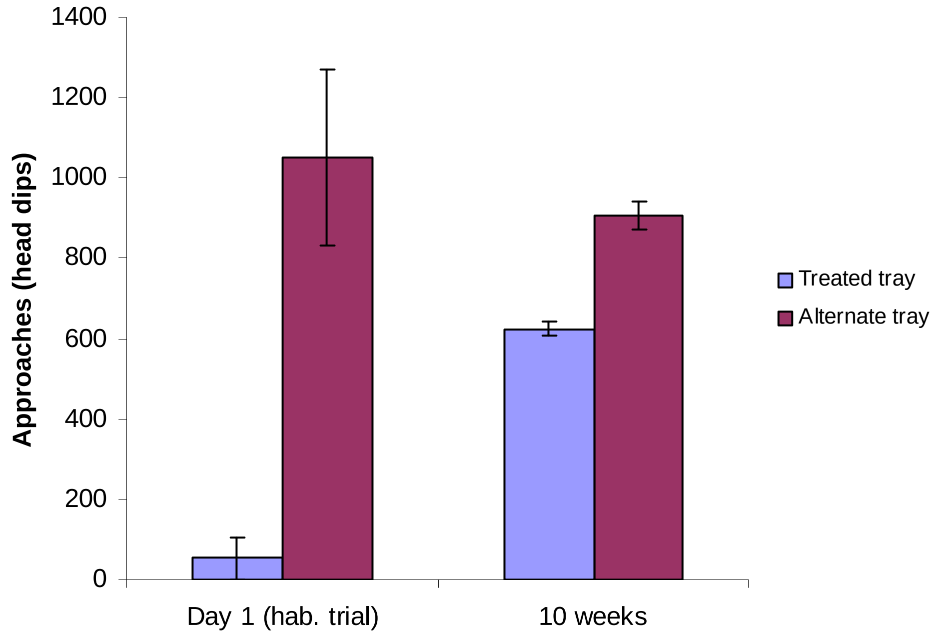

Figure 5.4 The number of approaches to the treated and control trays for the 10-weeks treatment and for Day 1 of the habituation trials (Chapter 4). Note: error bars represent 1 standard error.

5.4 Discussion

Following ten weeks of environmental exposure, Plant Plus retained the properties of a feeding repellent for *M. rufogriseus banksianus*. Food consumption and the number of approaches to feed trays by *M. rufogriseus banksianus* were significantly reduced when food was associated with Plant Plus aged for ten weeks in comparison to untreated food. The length of time that Plant Plus remains effective after application could not be elucidated due to contamination of data and methodological constraints, however, it is greater than ten weeks.

The longevity of Plant Plus in the field is an important consideration as it determines application regimes, cost efficiency and effectiveness (Coleman *et al.*, 2006). While the longevity of repellents varies both between repellents and scenarios (e.g. dependent on factors such as landscape and climate), most successful repellents that are topically applied are effective for approximately three months (Nolte, 2003). The lifespan of some repellents can be increased significantly through the use of slow release technologies including: mircoencapsulation of repellents (Mogul *et al.*, 1996; Boh *et al.*, 1999); impregnating the repellent into light sensitive foams and/or slowly degrading products (see Putman, 1997); or the use of slow-release devices (Sullivan *et al.*, 1990; Burwash *et al.*, 1998a; Kinley & Newhouse, 2004). The results of the longevity trial indicate that Plant Plus has an effective lifespan similar to other topically applied repellents that have been successfully used on other species, and the lifespan could be extended utilising microencapsulation or special release devices. The longevity of Plant Plus determined by this trial is sufficiently long to indicate that with further research and development, Plant Plus may be an economically viable wildlife management tool.

The longevity of animal repellents has usually been established during field trials (e.g. Montague, 1994; Santilli *et al.*, 2004; Baker *et al.*, 2005). However, assessing longevity in field trials is sub-optimal as there can be many confounding factors influencing the effectiveness of the repellents and field trials tend to be expensive. With improvements to the methods used in this trial, longevity could be established effectively and efficiently using captive methods. The longevity trial could be improved by: increasing independence of samples (using individual animals or sub-groups of animals); increasing replication; increased recovery times allowing extinction of choices and enabling full removal of contamination; and modifying the choice format to allow greater choice between treated and untreated sources (more alternative food sources). Increasing replication would also provide greater power for statistical analysis. Due to inadequate and inappropriate replication in this trial, statistical analysis was limited.

While the aims of the longevity trial were not fully accomplished, the longevity trial has confirmed the effectiveness of Plant Plus as an animal repellent and has indicted that the longevity of Plant Plus is greater than ten weeks. With improvements to the method, captive longevity trials may provide a cheap, useful alternative to field-based longevity trials.

Chapter 6

Field trials of an odorous repellent for macropods

Chapter 6 Field trials of an odorous repellent for macropods

6.1 Introduction

6.1.1 Background

Field trials to assess the effectiveness of Plant Plus are required, as the effectiveness of Plant Plus in repelling *Macropus rufogriseus banksianus* in captive situations (Chapters 2-5) does not directly relate to its effectiveness in the field. While repellents with good efficacy in captive situations are likely to be effective in a field situation, the efficacy of repellents in the field can not be extrapolated from data collected in captive situations alone (Nolte, 2003).

The importance of following up successful captive trials with field trials was highlighted by research conducted with repellent odours for *Rattus rattus* (Burwash *et al.*, 1998a, 1998b). Captive studies highlighted the effectiveness of 3,3-dimethyl-1,2-dithiolane (DMDT) and 2,4,5-trimetyl-Δ3-thiazoline (TMT) in reducing food consumption and altering the behaviour of *R. rattus* (Burwash *et al.*, 1998b). However, in field studies with the same repellent odours, no significant decrease in food consumption or consistent trends in behavioural responses could be detected (Burwash *et al.*, 1998a).

The effectiveness of repellents in the field has commonly been measured for browsing herbivores by assessing treated and untreated seedlings for damage attributable to browsing (Dietz & Tigner, 1968; Conover, 1984; Swihart & Conover, 1990; Swihart *et al.*, 1991; Morgan & Woolhouse, 1995; Santilli *et al.*, 2004). However, some field studies have utilised changes in animal density or behaviours (Boag & Mlotkiewicz, 1994; Wolff & Davisborn, 1997; Borowski, 1998b; Burwash *et al.*, 1998a; Van Der Ree & Nelson, 2002) or have monitored artificial feed stations (Bramley & Waas, 2001; Seamans *et al.*, 2002; Kinley & Newhouse, 2004; While & McArthur, 2006) to assess effectiveness of repellents. As *M. rufogriseus banksianus* predominantly graze and are not browsers, field studies assessing the response to repellents need to focus on animal densities, behaviours in response to repellents, or focus on establishing artificial feeding stations. No standard methods exist for such trials (Nolte & Mason, 1998).

Diet, feeding and feeding behaviour of macropods has been extensively studied (e.g. Lentle *et al.*, 1998; Evans & Jarman, 1999; Baxter *et al.*, 2001; Stirrat, 2002; Lentle *et al.*, 2003a; Lentle *et al.*, 2003b; Koch *et al.*, 2004; Telfer & Bowman, 2006). *Macropus rufogriseus* predominantly graze on monocotyledon grasses, however some browse is also consumed (Sprent & McArthur, 2002). A large proportion of time is spent feeding and participating in feeding related behaviour and it has been reported that female *M. rufogriseus banksianus* spend more time feeding than male *M. rufogriseus banksianus* (Coulson, 1999).

The use of direct (observation and radio tracking) and indirect (scat analysis, scat abundance and scat distribution) methods have been utilised in studies of free-ranging *M. rufogriseus* (Coulson, 1999; Higginbottom, 2000; Sprent & McArthur, 2002; While & McArthur, 2005). Recently, artificial feeding stations have also been utilised in the study of feeding and related behaviour for *M. rufogriseus rufogriseus* (While & McArthur, 2006).

6.1.2 Aims

The aim of the field trials was to assess if Plant Plus is an effective repellent for *M. rufogriseus banksianus* under field (non-captive) conditions. Specifically, the objectives of the field trials were to:

- Determine the ability of Plant Plus to reduce effective densities of *M. rufogriseus banksianus* in a road easement;
- Determine the effectiveness of Plant Plus in altering macropod feeding behaviour under field conditions; and

- Assess and relate the response of *M. rufogriseus banksianus* to Plant Plus under field conditions, to the response observed in controlled conditions (captive trials: see Chapters 2-5).

Two separate trials were conducted to address these objectives: a field-based density related trial; and a two-choice, feeding trial. The aim of the field-based density trial was to determine if the density of *M. rufogriseus banksianus* could be reduced in easements by applying Plant Plus to grazing areas (easements). The aims of the two-choice field based feeding trial were to determine if the results of captive trials could be replicated under field conditions and to assess if Plant Plus altered the feeding behaviour of *M. rufogriseus banksianus*.

6.2 Methods

The trials described in this chapter received ethics approval from the Animal Care and Ethics Committee of the Director General of New South Wales Agriculture (Approval Number 02/1926-3). The trials were conducted in accordance with the Australian Pesticides and Veterinary Medicines Authority (APVMA) under permit (PER 7250). Copies of the ethics approvals, APVMA permit and the National Parks and Wildlife Service permit are located in Appendix A.

6.2.1 Study site

Both field trials were conducted on the Tomago Sandbeds, near Medowie, NSW. The Hunter Water Corporation manages the area of land and a copy of the approval to use the land for the trials is located in Appendix A. The site was chosen for the field trials following a selection process conducted in July 2004 involving the inspection of several potential sites. Several characteristics and features were identified as essential in a study site and included:

- Presence of *M. rufogriseus banksianus*;
- Presence of a wide linear area suitable for wallaby feeding (equivalent to a road easement), adjacent to vegetation suitable for shelter/diurnal use by *M. rufogriseus banksianus*; and
- Relative homogeneity of topography, vegetation and habitat over the entire study site.

The selection process indicated that the Tomago site fulfilled these requirements. Three main areas of land were identified for use during the trial. Each area was based around private service roads (with very wide grassy easements) running through the forested areas of the Tomago Sandbeds (Figure 6.1).

The Pleistocene sands of the Tomago Sandbeds are coarse-grained and drain well (Geary, 2004). The study sites were centred on wide (~30 metre) easements that contained a variety of grasses suitable for grazing by *M. rufogriseus banksianus*. The adjacent vegetation communities surrounding the study sites were typical of coastal and/or dry sclerophyll forest, with dominant upper stratum flora inclusive of *Eucalyptus parramattensis decadens* (Earp's gum), *E. robusta* (swamp mahogany), *E. gummifera* (red bloodwood), *E. haemastoma* (scribbly gum) and *Angophora costata* (smooth-barked apple). The flora of the understorey included *Banksia aemula* (Wallum Banksia), *B. oblongifolia* (rusty Banksia), *Leptospermumn polygalifolium* (lemon-scented Tea-tree) and *Xylomelum pyriforme* (woody Pear). A detailed study of the growth and structure of the vegetation on the Tomago Sandbeds was undertaken during the 1990s and included a detailed site analysis and description applicable for the study sites utilised for this trial (see Fox *et al.*, 1996).

During the field investigation stage of the site selection process *M. rufogriseus banksianus* were sighted, confirming the presence of the species in the area. Consultation of the NSW National Parks and Wildlife Atlas revealed that sightings of the species were common, and that *M. giganteus* (eastern grey kangaroo) and *Wallabia bicolor* (swamp wallaby) were also common in the area (Department of Environment and Conservation, 2004). No sightings of *M. robustus* (wallaroo) had been recorded on Hunter Water Corporation land, but sightings had been recorded nearby.

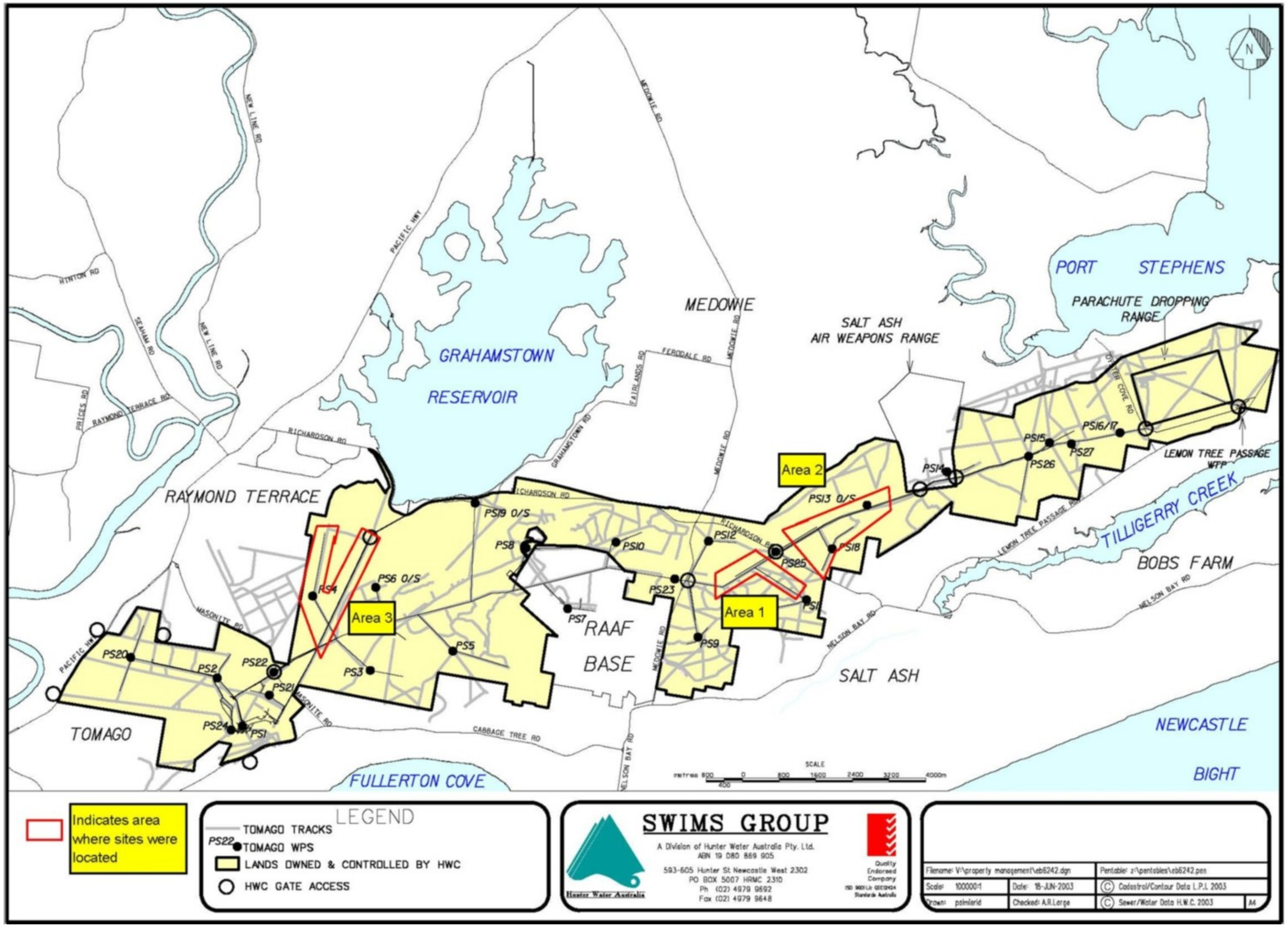

Figure 6.1 Map of field study sites (courtesy of Hunter Water Corporation).

6.2.2 Experiment 1: Density-based trial

A 'Before After Control Impact' (BACI) study was utilised to determine if the application of Plant Plus to grazing habitat (easement) could reduce densities of *M. rufogriseus banksianus* and other locally present macropods (*M. giganteus*, *W. bicolor*) in these habitats. The experiment was conducted between November 2004 and July 2005.

After the initial site inspection, 18 sub-sites were identified and selected for study. All sub-sites were located in Area 1 or Area 2 of the Hunter Water Corporation land (Figure 6.1). Sub-sites were separated by a distance of ≥200 m and were marked with a wooden stake and classified as either control or treatment using a stratified method to minimise area biases (equal proportion of control and treatment sub-sites in each area with random allocation).

At each sub-site, faecal plots were established utilising the methods discussed by Southwell (1989) to allow a macropod density index to be estimated from faecal accumulation. Each faecal plot was a fixed circular quadrat approximately 50 m^2 in size (4 m radius). On the first site visit, all faecal plots were cleared of faecal material while conducting a visual inspection of each site. The visual inspection was conducted by moving slowly around the centre of the plot, observing successive one metre wide strips to maximise the likelihood of detecting all scat material (Figure 6.2). One metre wide "searching edges" have been identified as the most effective and efficient way of looking for, and collecting scat samples (see Southwell, 1989 for review). All subsequent faecal collection was conducted using identical methods.

The rate of decay of *M. rufogriseus banksianus* faecal pellets has previously been established: however, rates of decay have varied significantly between studies and sites (see Southwell, 1989 for review). To assess if the decay of faecal matter would affect faecal accumulation over the collection periods (two weeks), four samples of fresh scat (each of six *M. rufogriseus banksianus* pellets) were placed in systematic, distinguishable patterns at different locations in the easement. The subsequent loss of faecal matter was noted over a nine-week period as presence or absence to determine rates of faecal loss per plot.

Macropod faecal material is very distinguishable and the key, descriptions and photographs detailed by Triggs (1996) allowed reliable identification to species level. Faecal pellets of *M. rufogriseus banksianus* and *M. giganteus* can be separated by their size and shape (Hill, 1978) while the pellets of *W. bicolor* are distinguishable by shape and texture. Faecal accumulation was monitored and a fortnightly collection period ensued. The numbers of pellets and also pellet-groups (a group of pellets deposited in one defecation, distinguishable by proximity and age) were counted per quadrat.

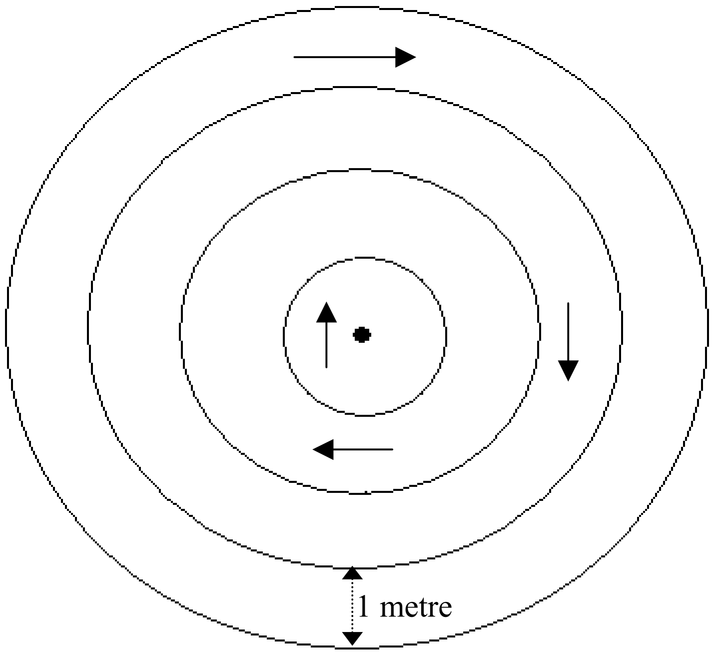

Figure 6.2 Diagrammatical representation of faecal plot sampling method. Four circular pathways, were visually inspected successively providing a 1 metre wide searching edge (faecal sample area ~ 50 m^2, treatment applied to sub-site area ~ 144 m^2: 12 m X 12 m).

Baseline faecal accumulation was assessed between November 2004 and June 2005 with a minimum of six fortnightly faecal collections at each sub-site. In June 2005, Plant Plus was applied at and around treatment sub-sites (12 m X 12 m) and faecal accumulation continued to be monitored fortnightly at all sites. Plant Plus was applied following the manufacture's instructions at a rate of 20 ml per square metre. The Plant Plus was sprayed on to the vegetation in the easement using a pressurised dispenser (Hozelock 5 l manual sprayer, No 4445). Reverse osmosis water was distributed at all control sub-sites under the same regime using an identical, uncontaminated sprayer.

6.2.3 Experiment 2: Choice-based field trial

The methods used for the choice-based field trial are loosely based on the methods described by Nolte & Mason (1998) and utilised in the captive trials described in Chapters 2, 4 and 5. The field-based choice trial was conducted in July and August 2005 and involved placing two artificial feeding stations, in each of the three areas outlined in Figure 6.1. In each area, the feed stations were situated on the same bearing and aspect, but separated by a distance of 20 m (the same distance between feed stations during habituation trials: Chapter 4). The feed stations were located four metres from the interface between easement and woodland. Each artificial feeding station consisted of a 1.2 m X 3.0 m X 2.0 m shelter (open only at one end) with a feed tray positioned at the closed end. The shelters were constructed from timber pickets and welded mesh, covered in a plastic tarpaulin. An infrared counting device (custom design and manufacture by Mudies Electronic Services Pty Ltd **http://www.mudies-es.com/**) was placed at the open end of each shelter. The counting device utilised an infrared beam directed horizontally across the entrance of the shelter, 0.3 m above ground level. The device recorded an event each time the beam was disrupted, although to avoid double counting, a period of 30 seconds would lapse after each record before a new event could be recorded. The devices were designed to count the number of visitations to the stations by macropods. The devices were tested before deployment to ensure accurate data collection. Data were collated by time into days and collected by linking a laptop computer to an output port every five days.

The choice-based field trials were undertaken on the easements at Medowie (Figure 6.3) following the methods outlined in Section 2.2.4 with the following exceptions:

- Plant Plus was the only repellent trialled and water was deployed as control;
- The deployment device was a secured, open, high density plastic bottle placed above the feed trays (containing 50 ml of either Plant Plus or water);
- In addition to pelleted kangaroo feed, chopped apple and a mixture of peanut butter, oats and honey were placed in the feed trays in large quantities and replenished every four to five days;
- Test subjects were free-ranging (not captive) macropods (*M. rufogriseus banksianus* and *M. giganteus*)
- Passive infrared counting devices were used to calculate macropod approaches instead of video surveillance; and
- Analysis was to be performed comparing pre-treatment, treatment and post treatment data.

Following the completion of the choice-based feeding trials, the feeding stations were removed and visual observations were made of macropods in clearings to ensure their welfare was not compromised by the removal of feed stations.

Figure 6.3 Photograph of easement and adjacent woodland near the feed stations located in Area 2.

6.2.4 Data Analysis

The data collected in experiment one (faecal pellets and pellet-groups) were checked for anomalies. The relationships between herbivore density and pellets and/or pellet-groups tend to be non-linear and often follow a negative binomial distribution (e.g. White & Eberhardt, 1980). Models to assess the distribution of the datasets were estimated using maximum likelihood estimators for k and p (the parameters of a negative binomial distribution) utilising XLSTAT 2007. Faecal data were transformed into overall ranks (for each of the pellet-group and individual pellet datasets) and analysed utilising a partitioned, non-parametric 2 X 2 analysis, following the methods of Puri & Sen (1969) and further adapted by Thomas *et al.* (1999).

The data collected from the choice-based field trial (approaches to feed stations, consumption of food) were checked for anomalies. A within area comparison in the utilisation of the food resource over the pre-treatment, treatment and post treatment periods was the focus of the primary analysis. However, a comparison between areas could have been utilised. The variables for these comparisons were macropod visitation (as logged by infrared monitoring device), although the amount of food consumed (mass) and the presence of scats within feeding stations could have been utilised.

6.3 Results

6.3.1 Experiment 1: Density-based trial

Due to easement maintenance (including the burning of nearby vegetation or grass slashing) some fortnightly scat collection periods conducted before the application of treatments were excluded due to processes effecting faecal accumulation. Unfortunately, due to unforseen/emergency maintenance work conducted over the entire easement shortly after treatment application, only one sampling period was conducted following the application of Plant Plus. All faecal sampling quadrats were destroyed by the maintenance work.

The decay of faecal pellets was observed over a nine-week period in which minimal loss was noted (Table 6.1). Plot number two was the only plot in which faecal material disappeared, with the first occurrence (within the first week) suspected to be from disturbance to the site by animal movement (adjacent scats in the same plot were observed to have moved slightly). The only other absence of scat was noted after five weeks.

Table 6.1 Observations of macropod faecal pellet over time at 4 sites in the study area.

	Number of pellets remaining per plot			
	Plot number			
	1	*2*	*3*	*4*
December 22: Scat Placement	6	6	6	6
December 29: 1 week	6	5	6	6
January 12: 3 weeks	6	5	6	6
January 26: 5 weeks	6	5	6	6
February 9: 7 weeks	6	4	6	6
February 23: 9 weeks	6	4	6	6

The total number of macropod faecal pellets collected during the seven collection periods for the 18 sites utilised in the trial was 2827. Over 63% of the faecal material collected was identified as *M. rufogriseus banksianus* faeces, 36% was identified as faeces of *M. giganteus*, with less than 1% either from *W. bicolor* or unidentifiable. Due to the low amount of faecal material collected and the similarities in biology between the two major species, faecal data from all macropods were combined for analysis.

The number of faecal pellet-groups collected varied greatly both between and within sites (Figure 6.4). The distribution of all pellet-group scores approximates a negative binomial distribution (k=1.1, p=9.6) with a mean of 10.4 and variance of 110.2 (Figure 6.5). Due to insufficient and unequal replication, models could not estimate the distribution of each treatment (control–before, control-after, treatment-before, treatment-after).

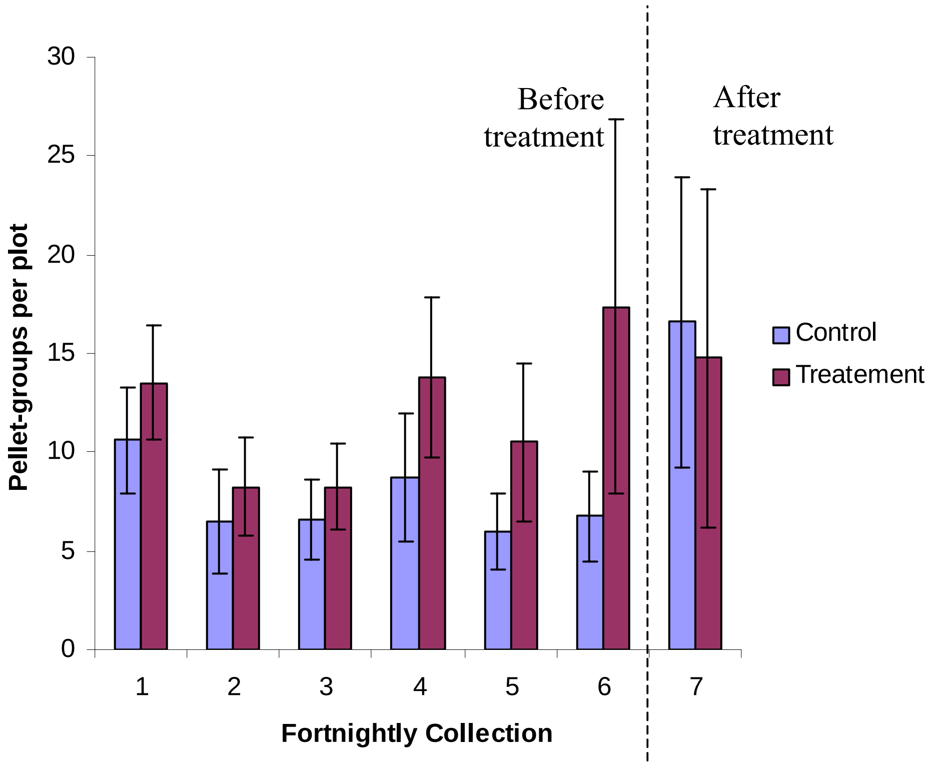

Figure 6.4 The mean number of faecal pellet-groups collected at control and treatment plots each fortnight. Error bars indicate one standard error. Treatment with Plant Plus occurred after faecal collection number 6.

The number of faecal pellets collected varied greatly within plots (Figure 6.6). For all sites over the entire collection period the number of individual pellets collected per plot ranged from 0 to 173 and approximated a negative binomial distribution (k=0.8, p=22.5) with a mean of 18.4 and variance of 432.1 (Figure 6.7). Due to unequal sample sizes and insufficient replication, models could not predict the distribution of data for treatment groups.

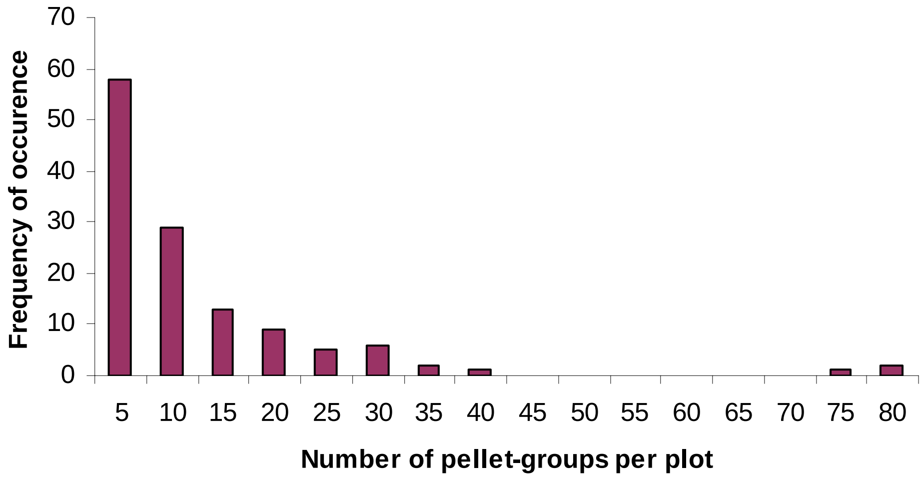

Figure 6.5 Histogram of the pellet-groups detected per plot for all collections. Distribution approximates a negative binomial distribution (k=1.1, p=9.6, M=10.4, σ^2=110.2)

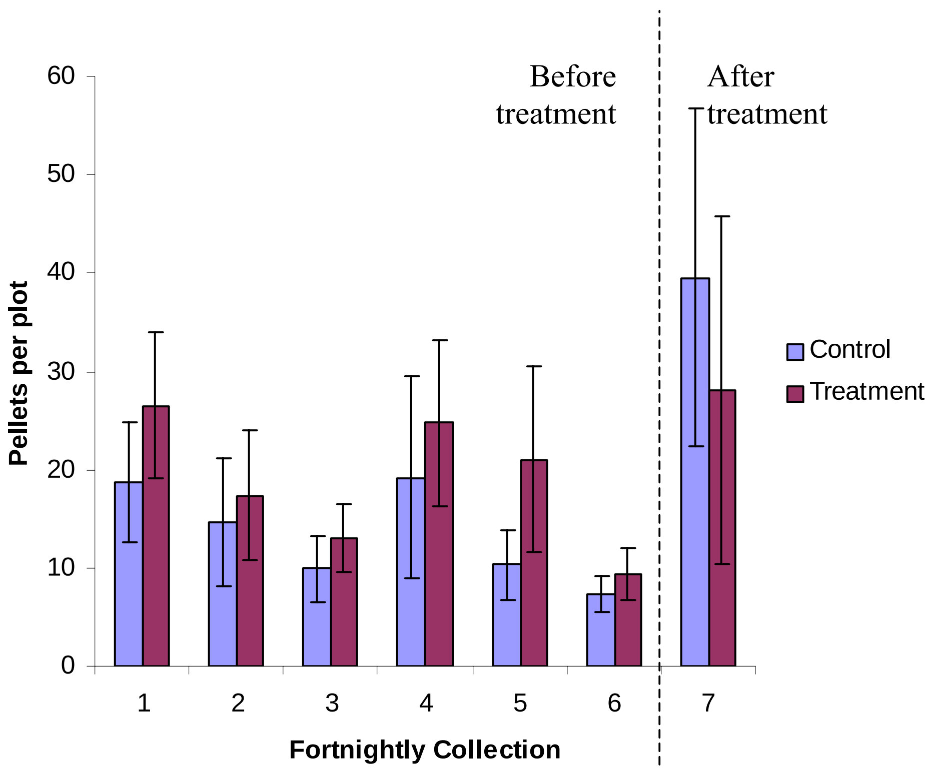

Figure 6.6 The mean number of faecal pellets collected at control and treatment plots each fortnight. Error bars indicate one standard error. Treatment with Plant Plus occurred after collection number 6.

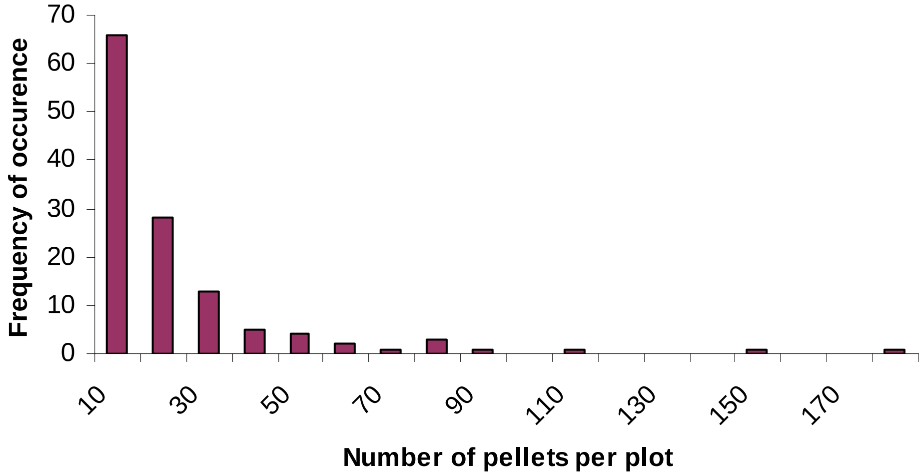

Figure 6.7 Histogram of pellets detected per plot for all collections. Distribution approximates a negative binomial distribution (k=0.8, p=22.5, M=18.4, σ^2=432.1).

A 2 X 2 non-parametric analysis, utilising rank scores to investigate the effect of treatment (Plant Plus or water) and time (before or after treatments were deployed), revealed no treatment, time or interaction effects were apparent for either pellet-groups or pellets (Table 6.2). The trends detected for pellet-groups (Figure 6.8) and pellets (Figure 6.9) were similar, with variance relatively large for both data sets. It is also noted that for both pellet-groups and pellets, that the collection after application of treatments is the only time when the treatment data ranks less than the control data. This trend was also apparent in the raw data for pellets and pellet-groups (Figures 6.4 and 6.6 respectively).

Table 6.2 Results of a 2 X 2 non-parametric analysis of ranks to investigate the effectiveness of Plant Plus in reducing accumulation of faecal pellets or pellet-groups. The analysis was performed following the methods of Puri & Sen (1969) and further adapted by Thomas *et al.* (1999).

	Treatment (Control/Impacts)	**Time (collections)** Note: Partitioned to before and after application of treatments	**Interaction (treatment X time)**
Pellet-groups	L(1,16)=0.0002, p>0.05	L(1,16)= 0.02, p>0.05	L(1,16)=0.16, p>0.05
Pellets	L(1,16)=0.00013, p>0.05	L(6,11)=0.05, p>0.05	L(6,11)=0.23, p>0.05

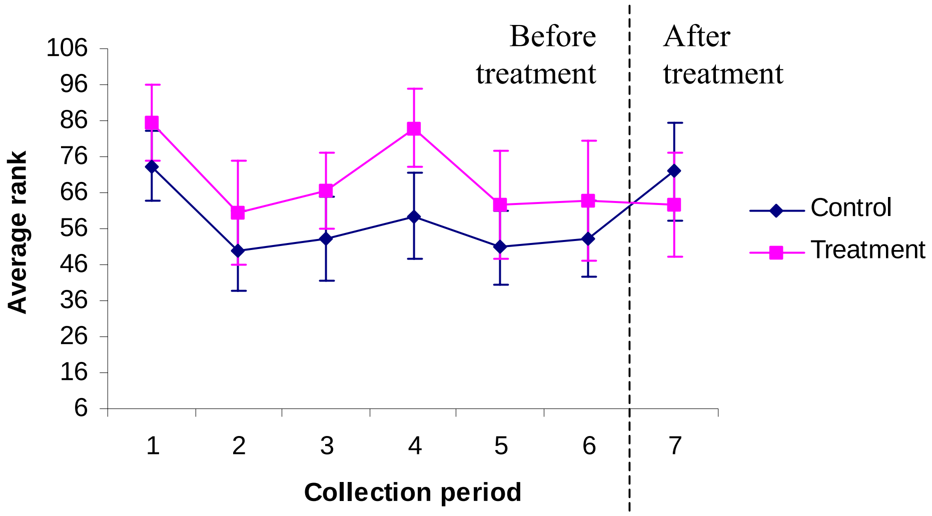

Figure 6.8 Trend of faecal pellet-group data when ranked for analysis. Note: error bars indicate 1 std error.

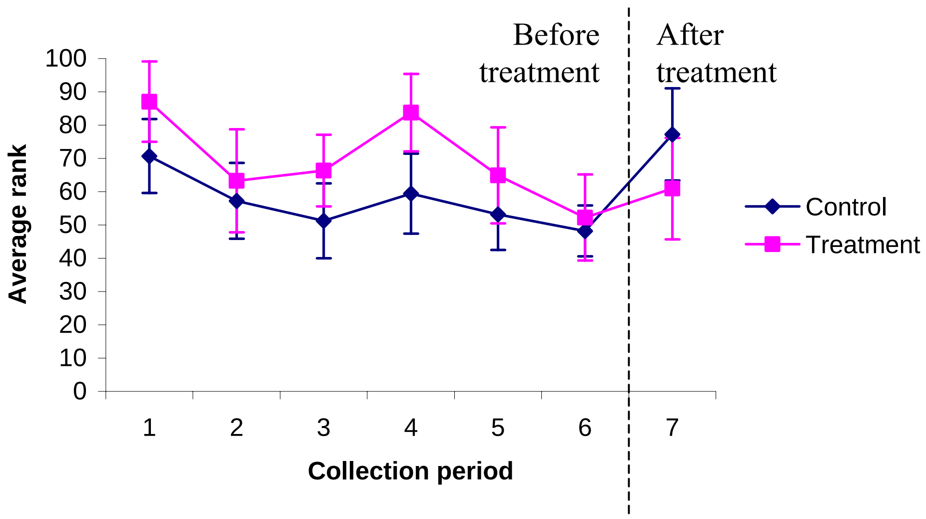

Figure 6.9 Trend of faecal pellet data ranked for analysis. Note: error bars indicate 1 std error.

6.3.2 Experiment 2: Choice-based feeding trial

The two feed stations in each of three areas received a total monitoring effort of 140 nights, each area receiving at least 20 nights of monitoring to both feed stations simultaneously (Figure 6.10). On only four occasions, visitations to stations were greater than five per night (136 observation of ≤ five, including 98 occurrences of zero visitation). During this time various amendments to the feed station construction and feed mixture were made in an attempt to increase visitation. After repeated attempts with the available materials, the trial was suspended during the pre-trial stage due to lack of visitation and associated data. No Plant Plus was applied during these trials.

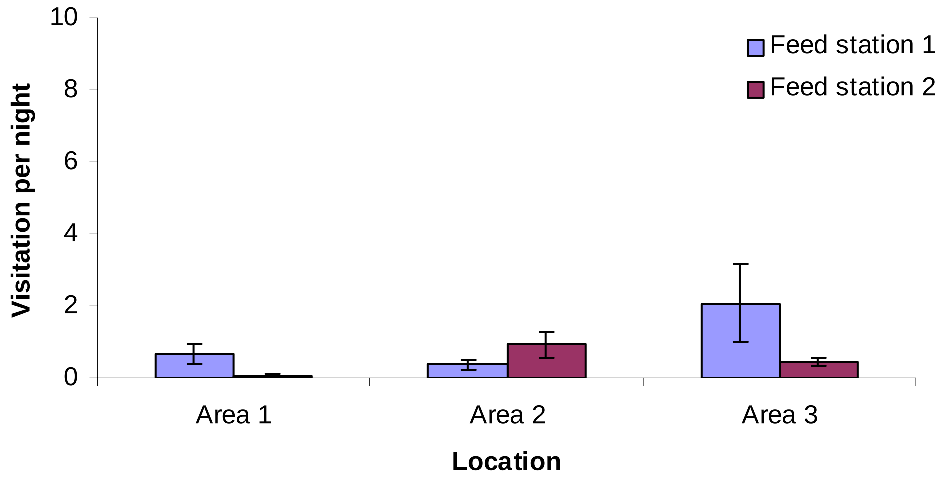

Figure 6.10 Average visitation (per night) to the feed stations located in Areas 1, 2 and 3. Note: Error bars indicate one standard error

6.4 Discussion

In captive situations, Plant Plus has effectively repelled *M. rufogriseus banksianus* from feeding stations (Chapters 2-5). The results of these field trials were inconclusive and did not elucidate if Plant Plus could reduce effective densities or alter feeding behaviour of macropods in a road easement under field (e.g. non-captive) conditions. The inconclusive nature of these trials also prevents a comparison of the behaviour of *M. rufogriseus banksianus* in response to Plant Plus between captive and field situations.

The reasons for the inconclusive results of the two field experiments are different, but are both related to the lack of data. The choice-based feeding trial was unsuccessful in achieving its aims due to an inability to reliably attract macropods to feeding stations. The density related trial was flawed due to the large background variance in the density index (based on faecal sampling) and a short post-treatment monitoring period (Figure 6.4 and Figure 6.6) due to unexpected maintenance works conducted on the field sites.

The sampling of faecal material has been used extensively to monitor densities of herbivores (see Neff, 1968), including macropods (see Southwell, 1989). The use of pellet-groups (as opposed to individual pellets) appears to be a more reliable method of estimating a density index for deer and other large herbivores (Neff, 1968). However, the use of individual pellets is common with macropods (e.g. Caughley, 1964; Floyd, 1980; Taylor, 1980; Hill, 1981; Perry & Braysher, 1986; Arnold & Maller, 1987; Vernes, 1999; Bender, 2003; Bulinski & McArthur, 2003). An investigation of faecal pellet census methods with a population of *M. rufogriseus banksianus* of known density concluded that both individual pellets and pellet-groups could both be used to accurately determine animal densities (Johnson & Jarman, 1987). The similarities in the pellet and pellet-group data sets collected in the road easement for the density experiment supports the utility of both pellets and pellet-groups as an index for *M. rufogriseus banksianus* density.

With a large number of small (10 m^2) circular plots, Hill (1981) determined the population of *M. giganteus* in Durikai State Forest (Queensland) on two occasions with minimal within site variance in the estimates. However, it was concluded that fewer, larger plots could be utilised to more accurately and efficiently assess between site variance and increase reliability with minimal impact on within site variance. However, the larger plots (50 m^2) utilised in the density experiment exhibited large variance both within and between sites.

Vernes (1999) reported accurately estimating densities of *Thylogale stigmatica* (red-legged pademelon) using faecal count methods at four sites on the Atherton Tablelands (Queensland). Following a pilot study, 99 clustered 3 m^2 permanent plots were utilised at each site with a faecal accumulation period of one month. The methods of calculating the number of plots required were similar to those used by Johnson & Jarman (1987) who studied a population of *M. rufogriseus banksianus* on a 160 ha site and accurately predicted density by measuring faecal accumulation (the methods were designed to estimate density within 25% of the true mean). An initial survey revealed highly skewed data and resulted in the increase of plot sizes (from 3 m^2 to 9 m^2) and the number of plots surveyed. Faecal plots were cleared two months prior to collection. For the density related experiment, initial survey effort (area of land surveyed) was similar to Vernes (1999). However, due to maintenance work, vandalism or repeated zero counts, some plots were excluded from the trial, reducing the effective survey effort.

A major difference between the methods used in this trial and those used by Johnson & Jarman (1987) and Vernes (1999) was the length of time for faecal material accumulation. The two-week faecal accumulation period was utilised so any potential short-term effects of Plant Plus could be detected. A similar faecal accumulation period was used by Bender (2003) who also needed to detect short-term density changes. However, the accumulation periods used by other successful studies have been longer (e.g. Floyd, 1980; Hill, 1981; Johnson & Jarman, 1987; Vernes, 1999; Bulinski & McArthur, 2003). More sampling plots and longer accumulation periods may have assisted in reducing the variance of the datasets, as the decay or loss of pellets would not have influenced the accuracy of longer accumulation periods (Table 6.1).

Bender (2003) utilised faecal count methods to assess the response of free-ranging *M. giganteus* to an acoustic repellent device. Captive trials had indicated that the acoustic device was inaudible to the subjects and ineffective. No treatment effect was detected by an analysis of variance conducted on faecal data (cube root transformed) from the field trial. However, it is noted that faecal pellet density at treatment sites was consistently half the mean of control sites. The non-significance of results reported by Bender (2003) may have been due to residual variance similar to the results reported for this density related trial. An alternative method of analysis that could have been utilised by Bender (2003) to compare datasets makes use of the parameters of contagion (k) and mean ($\underline{M}$) for the negative binomial distribution (methods described by White & Eberhardt, 1980; White & Bennetts, 1996). Unfortunately, this method could not be used for the faecal density trial conducted at Medowie due to the unequal sample group sizes and low replication that were exacerbated by the unexpected shortening of trials due to easement maintenance. However, the methods utilising the negative binomial distribution (described by White & Eberhardt, 1980; described by White & Bennetts, 1996) would have been expected to be useful as overall faecal accumulation in plots at Medowie followed a negative binomial distribution (Figures 6.5 and 6.7).

The decision to collate macropod scats and not calculate a density index for each species was necessary due to the low number of pellets encountered. This decision is likely to have avoided observer error in identification of macropod scats which has been identified as an area of concern (Bulinski & McArthur, 2000). Johnson & Jarman (1987) found that in initial surveys, faeces of sub-adult *M. giganteus* were often misidentified as pellets from *M. rufogriseus banksianus*. Johnson & Jarman (1987) also reported that collating pellet-groups counts gave an accurate density index of the two species combined.

While the density of macropods has not been established for the study site, calculating the average pellet count per plot per night for the entire study, and utilising the defecation rate for *M. rufogriseus banksianus* published by Johnson & Jarman (1987) of 311 defecations per animal per day, a density estimate for the site is approximately 0.8 animals per hectare. However, the actual density is likely to be much lower as it has been observed that macropods tend to defecate more when in feeding areas (Caughley, 1964; Hill, 1978; Johnson *et al.*, 1987) and this study only sampled feeding habitats. This figure is also unreliable due to wide temporal and spatial variations in defecation rates (Caughley, 1964; Hill, 1978; Perry & Braysher, 1986; Johnson *et al.*, 1987). While the density estimate for the study site is similar to the density estimate of the site studied by Johnson & Jarman (1987), it is a relatively low density when compared to Perry & Braysher (1986) and Vernes (1999) who successfully estimated macropod densities at 4.9 animals per hectare and 2.3 and 11.7 animals per hectare respectively.

There was an apparent trend evident in both the pellet-group and individual pellet data in which the only collection period when the mean of the treatment plots was lower than the control plots was after the application of Plant Plus (Figures 6.4, 6.6, 6.8 and 6.9). All captive-based trials (Chapters 2-5) indicated that *M. rufogriseus banksianus* significantly responded to Plant Plus by reducing visitation to feeding areas as well as food consumption and a similar response in the field could be responsible for this trend. However, the trend was not detected as significant by the analysis. Unfortunately, it was not possible to perform Bernoulli equations to assess the probability of the trend occurring, as collection periods are repeated measures and not independent observations. The non-significance of results may be due to the flawed sampling design, inadequate replication and/or the relatively conservative non-parametric analysis techniques performed. However, further investigation into the apparent trend and possible reasons associated with the trend should be pursued.

Choice-based feeding trials utilising artificial feeding stations have been used successfully to determine the effectiveness of repellents in field situations (e.g. Seamans *et al.*, 2002; Kinley & Newhouse, 2004). The effects of three potential area repellents on free-ranging *Odocoileus hemionus* (mule deer), *O. virginianus* (white-tailed deer) and *Cervus elephus nelsoni* (elk) were tested in Canada utilising artificial feed stations (Kinley & Newhouse, 2004). The artificial feed stations were open ended pens, constructed from wire and rebar (steel rods) which were built after bait (mixed alfalfa-grass hay) had attracted animals to the site for two consecutive nights. The stations also utilised infrared motion detectors (linked to camera) to detect animal presence. These structures differed to the ones deployed in the choice-based feeding trial as they did not require the food to remain dry and did not have a cover to keep rain or snow out. Due to the size differences in the target species, dimensions of stations also differed.

Seamans *et al.* (2002) constructed feed stations from plastic snow fence to attract *O. virginianus*. Feed stations were open-ended and contained a feed tray containing corn kernels. Infrared detectors were used to detect visitation. The trial was conducted during a season of low resource availability to enhance visitation to the feed stations. The numbers of visitations to feed stations were monitored pre-treatment, during treatment and post treatment to investigate the repellent effects of *Canis latrans* (coyote) hair. The trial confirmed the effectiveness of *C. latrans* hair as an area repellent for *O. virginianus*.

The main methodological differences between the choice-based trial attempted at Medowie and the trials conducted by both Seamans *et al.* (2002) and Kinley & Newhouse (2004) relate to feed station construction and food placement. The feed station materials could have affected visitation in several ways. Due to the high wind speed common for Medowie (B. Gumb, Hunter Water Corporation, pers. comm.) it is possible that movement and resultant noise of materials associated with the feed station (especially of the tarpaulin cover used to prevent moisture spoiling the food) could have reduced animal visitation. Excessive movement of the tarpaulin (creating noise) was observed on several occasions. The use of more durable food (e.g. sorghum or lucerne) could have reduced the need for the weather proof covering, which may have led to increased visitation.

The success of artificial feed stations to monitor feeding preferences of *M. rufogriseus rufogriseus* has recently been reported (While & McArthur, 2006). The methods involved using many small clear plastic feed trays that contained red sorghum as a food source in a pine-bark matrix. Consumption of the sorghum (mass removed) and the abundance of scat around feed trays were used as measures of visitation. This method did not allow direct observations and the consumption variable may have been confounded by the presence of other species. However, the indirect observations collected (mass of food removed and abundance of scats observed) were correlated and appeared to provide a reliable index of animal abundance. The methods reported by While & McArthur (2006) may provide a useful approach to assesses the effectiveness of Plant Plus with free-ranging *M. rufogriseus banksianus*.

The effectiveness of Plant Plus was not elucidated from these field trials. A number of methodological constraints have been identified. The importance and utility of successfully conducting the trials remain as the effectiveness of Plant Plus on free-ranging *M. rufogriseus banksianus* is untested and provides a hurdle for its future use in wildlife management. Many improvements to the density related trial could be made and include:

- Study areas containing higher densities of target species;

- Larger survey plots
- Longer accumulation periods for faecal materials (e.g. ≥ one month);
- Greater post treatment monitoring period (e.g. ≥ three temporal samples); and
- Utilisation of pilot studies to calculate required sample sizes and plot sizes.

Recommended improvements to the choice based trial include:

- Study areas containing higher densities of target species;
- Trial period to commence when resource availability is low;
- Baits to contain more attractive foods, less vulnerable to spoil (e.g. lucerne, red sorghum);
- Use of temporal replication in addition to spatial replication; and
- Use of open feed trays and utilisation of indirect measures (food consumption, scat abundance) and/or the use of sturdier materials for feed station construction (e.g. no potentially moveable or noisy parts).

Chapter 7

Synthesis

7 Chapter 7 Synthesis

7.1 General Discussion

Plant Plus, a synthetic compound based on the chemistry of dog urine, was an effective repellent for captive *Macropus rufogriseus banksianus* (red-necked wallaby). Plant Plus was the most effective repellent formulation tested by this research, capable of successfully reducing food consumption, visitation to feeding areas and the number of animal movements through a scent-fence. *Macropus rufogriseus banksianus* did not habituate to Plant Plus over a six week period and Plant Plus appears to remain effective following exposure to ambient environmental conditions for at least 10 weeks. The effectiveness of Plant Plus as a repellent for *M. rufogriseus banksianus* under field conditions was not established and the ability for Plant Plus to reduce roadkill or to reduce the number of macropods in road easements could not be determined due to methodological limitations and extraneous factors.

An initial evaluation of four potential repellents for *M. rufogriseus banksianus* utilising choice-based feeding trials (Chapter 2) revealed feeding was significantly affected by the presence of two repellents: Plant Plus; and a formulation consisting of homogenised chicken eggs. A commercial repellent (SCAT® Bird and Animal Repellent) was found to have no effect on the variables measured during the trial, while the fourth compound trialled (Δ^3-isopentenyl methyl sulfide: IPMS) had an intermediary effect, close to statistical significance for the variables examined.

During the review of potential repellents for use in the pilot screening trials, a fifth compound (3,3-dimethyl-1,2-dithiolane: DMDT) was identified as a potential repellent for *M. rufogriseus banksianus*. However, this compound was not tested due to difficulties in the synthesis of the chemical. If a reliable source or method of manufacture can be established for DMDT, further screening of this compound for its repellent properties is recommended.

The lack of response by *M. rufogriseus banksianus* to SCAT® Bird and animal repellent was unexpected as the formulation has exhibited repellent properties with *Trichosurus vulpecula* (common brushtail possum: Cooney, 1998) and is similar to many other commercially available animal repellents (e.g. the new formulation of Rudduck's Dog and Cat repellent, Rudducks USA INC. Naples, Florida; Poss Off, Beat-A-Bug Corporation, Darlington, Western Australia; and D-Ter® Bird and Animal repellent). The pilot screening trial results indicated that further trials with SCAT® Bird and animal repellent were unwarranted as it had no repellent characteristics for use with *M. rufogriseus banksianus* and it is unlikely to be effective with other macropod species or be useful in roadkill mitigation.

IPMS has been previously trialled as a repellent for several species with mixed results (see Lindgren *et al.*, 1995). IPMS has been effective in reducing browsing by captive *T. vulpecula* (Woolhouse & Morgan, 1995) and deterring *Lepus americanus* (snowshoe hares) from feeding (Sullivan & Crump, 1986). Similarly to the pilot screening trials, browsing by *Wallabia bicolor* (swamp wallaby) was not reduced by IPMS in initial trials conducted in Victoria (Montague *et al.*, 1990). Due to the modest results of these pilot trials and some ambiguous results for IPMS previously reported, IPMS should not be dismissed as a repellent for macropods and further trials with IPMS and other macropod species would be worthwhile. However, the results of the pilot screening trials indicate that both Plant Plus and the egg formulation are better candidates to be effective repellents with *M. rufogriseus banksianus*.

A barrier trial (Chapter 3), where subjects were required to move through a scent-fence to access food, was conducted utilising the Plant Plus and egg formulations that exhibited repellent properties in the pilot screening trials. The barrier trial aimed to assess the strength of repellents and to determine if the products displayed area repellent characteristics. These characteristics are important if repellents are utilised in the field as they determine how a repellent can be used (food deterrent, movement inhibitor, barrier etc.). Plant Plus significantly reduced the number of movements by *M. rufogriseus banksianus* through a scent-fence, indicating that Plant Plus has the properties of an area repellent. The Plant Plus scent-fence was most effective in the hours immediately after application, with the effect size reducing within the 24-hour trial period. The scent-fence constructed from the egg formulation did not significantly affect movements of *M. rufogriseus banksianus* at any time period or overall.

Chicken Eggs, fermented chicken eggs and synthetic fermented egg have been identified as effective short-term repellents for several herbivores including *Cervus elaphus nelsoni* (elk: Andelt *et al.*, 1992), *Odocoileus sp*. (deer: Palmer *et al.*, 1983; Andelt *et al.*, 1991), and *T. vulpecula* (Eason & Hickling, 1992; Woolhouse & Morgan, 1995). Additionally, several effective deer repellents are based on compounds found in chicken eggs (e.g. MGK Big Game Repellent® and Deer Away®, see Bullard *et al.*, 1978; Melchiors & Leslie, 1985; White & Blackwell, 2003). During initial screening of repellents for *W. bicolor*, Montague *et al.* (1990) reported that neither egg or synthetic fermented egg significantly reduced browsing.

The pilot screening trial (Chapter 2) utilised a choice-based format, sensitive in the detection of repellence and was successful in detecting a response to the egg by *M. rufogriseus banksianus*. However, the barrier trials (Chapter 3) were analogous to a no-choice trial, which are useful in testing the strength of repellents (see Nolte & Mason, 1998). The aversive response to egg by *M. rufogriseus banksianus* in the pilot screening trials, coupled with the lack of response to egg observed in the barrier trial may indicate that either egg is only a topical repellent (as opposed to an area repellent), or that the response of *M. rufogriseus banksianus* is only weak in nature. Due to the presentation method of the egg during the pilot screening trials, and the response noted, it is likely that the egg was functioning as an area repellent. Therefore, the lack of response noted in the barrier trials with egg is indicative of a weak repellent. However, further investigation is required to determine the reasons why egg was not effective in the barrier trial. As Plant Plus exhibited properties of an area repellent and also induced a stronger response in *M. rufogriseus banksianus* than egg, focus was placed on further determining the repellent effects of Plant Plus for *M. rufogriseus banksianus*.

Plant Plus has induced anti-predator responses in two other species of macropod (*M. parma* (parma wallaby) and *Thylogale thetis* (red-necked pademelon); Ramp *et al.*, 2005) and also in *Oryctolagus cuniculus* (European rabbit) and *T. vulpecula* (Morgan & Woolhouse, 1995; Woolhouse & Morgan, 1995). Dog urine (Plant Plus is based on the fatty acids and sulfurous compounds in dog urine and is described as synthetic dog urine: Dr Thomas Montague, Roe Koh and Associates Pty. Ltd., pers. comm.) has elicited repellent or anti-predator effects for macropods including *W. bicolor* (Montague, 1994) and *M. fuliginosus* (western grey kangaroo; Parsons *et al.*, in press). The avoidance response of *M. rufogriseus banksianus* to Plant Plus is also likely to be an anti-predator strategy with Plant Plus mimicking a predator odour.

Habituation to fear inducing and predator odours is a major disadvantage for their use as repellents in wildlife management (Mason *et al.*, 2001). The efficacy and benefit of using a repellent in a management situation is reliant on the prolonged effectiveness of the repellent in the field. Habituation by target species is a major determining factor of a repellent's prolonged effectiveness as rapid habituation will lead to the loss of initial effectiveness that may not return (Apfelbach *et al.*, 2005).

Habituation to non-reinforced predator cues has been postulated to be rapid (McGregor *et al.*, 2002). However, habituation to aversive stimuli that causes avoidance behaviour is slower than habituation to disturbance (File *et al.*, 1993). During the assessment of habituation to Plant Plus by *M. rufogriseus banksianus* (Chapter 4), avoidance of Plant Plus did slightly decrease over a six-week period, although strong avoidance of Plant Plus remained. Food consumption in the presence of Plant Plus did not significantly increase during the six-week period, but a weak trend indicating a possible increase (with small effect size) in food consumption was noted. Habituation to Plant Plus was not rapid and *M. rufogriseus banksianus* continued to avoid visiting and feeding from feed stations associated with small quantities of Plant Plus after six weeks.

The avoidance of Plant Plus by *M. rufogriseus banksianus* is typical of a reaction to aversive stimuli and habituation was slow and minimal. Habituation to aversive stimuli is dose dependent and related to odour strength and frequency of stimulus presentation (habituation is more rapid to weak odours, frequently encountered odours and/or low concentrations of odours: Thompson & Spencer, 1966; Wallace & Rosen, 2000; Takahashi *et al.*, 2005). Due to the size of the enclosures, test subjects were constantly exposed to Plant Plus during the captive trials, yet minimal habituation was detected and Plant Plus was still effective after six weeks. In a practical management plan for wildlife control, it is reasonable to assume that encounters by animals with the repellent would be fewer, leading to an even slower rate of habituation. Gilsdorf *et al.* (2003) recommended several strategies for further minimising stimuli encounters (including periodic or animal activated release of stimuli) that could decrease the rate of habituation. Furthermore, an integrated pest management plan for macropods that incorporated control measures at a range of ecological scales (landscape, habitat, home-range, feeding patch and individual food items) may be more successful, as control measures affecting different scales may have an additive effect (see Miller *et al.*, 2006), further increasing the effectiveness of control measures and decreasing the frequency of encounters with repellents.

Either strengthening the concentration of Plant Plus (it is currently available at twice the concentration utilised by these trials) or increasing application quantities may also decrease the habituation detected in the captive trials, particularly if the application method does not increase the frequency of repellent encounters. Further captive trials to determine the dose dependant rate of habituation are required. Investigation into application methods that minimise stimuli encounters while increasing odour strength at the targeted area is also recommended.

Product-related longevity of a repellent is important as it determines application regimes and methods, and is important when assessing the costs associated with management plans involving repellents (Coleman *et al.*, 2006). Most topically applied repellents are only effective for up to three months, dependent on weather conditions (Nolte, 2003). The results of the Plant Plus longevity trial conducted with captive *M. rufogriseus banksianus* (Chapter 5) are consistent with this time frame and may indicate that a quarterly application regime would be suitable for year round usage in a field based application. As Plant Plus also has area repellent properties (Chapter 3), it may be possible to extend the product-related longevity by utilising application technologies. Such technologies include: micro-encapsulation, which allows impregnation of carrier devices (textiles, plastics, metals etc.) with repellents (Mogul *et al.*, 1996; Boh *et al.*, 1999); the use of slow-release deployment devices (urethane, PVC or rubber vials and containers: Sullivan *et al.*, 1990; Burwash *et al.*, 1998a; Kinley & Newhouse, 2004); and photosensitive foams (see Putman, 1997). The product-related longevity of Plant Plus is sufficient for targeted control programs with specific short-term objectives and with further research and development, may be useful in fulfilling longer-term objectives.

The assessment of Plant Plus as a repellent with free-ranging macropods is an important step in developing repellent technology for use in any management situation as the effectiveness of repellents in the field can be substantially different to the effectiveness of repellents in captive situations (Nolte, 2003; Apfelbach *et al.*, 2005). Unfortunately, the field trials that were conducted to assess the effectiveness of Plant Plus with free-ranging *M. rufogriseus banksianus* and *M. giganteus* (eastern grey kangaroo) failed in their objectives due to methodological limitations and constraints (Chapter 6). Background variance of all variables collected was much higher than expected and replication was insufficient to reliably detect any trends or effects. Using only slight modifications to the methods of the field trials, it is expected that the effectiveness of Plant Plus with free-ranging *M. rufogriseus banksianus* and the utility of Plant Plus in reducing numbers of macropods in road easements could be successfully determined. Combining the methods of the two trials that were attempted, by assessing animal densities and feeding behaviours around artificial feeding stations may be effective as these methods have recently been used in Tasmania with *M. rufogriseus rufogriseus* (Bennett's wallaby) to determine factors associated with feeding choices (While & McArthur, 2006). Increased spatial and temporal replication and selection of a high density population and/or resource-depleted site may improve trial success.

Apfelbach *et al.*(2005) reported that most field studies of predator odour-based repellents have focused on monitoring three types of behavioural effects on a target species: alteration in activity patterns (e.g. does the time or location of feeding change); reduction in non-defensive behaviours (e.g. deterring feeding); and shifts in habitat (e.g. movement of feeding ranges or home-ranges). The captive trials focused on reductions in feeding, however, the mechanisms behind the reductions observed were not investigated. As overall feeding was largely unchanged during trials (only feeding from treated areas was reduced) it is postulated that the results represent a change in activity patterns not a reduction in non-defensive behaviour. However, there was some evidence that a reduction in non-defensive behaviour occurred during the scent fence trial (Chapter 3), where a reduction in movement was detected. Further investigation of the mechanisms involved in repelling *M. rufogriseus banksianus* and other macropods, and the elucidation of the properties of Plant Plus would provide a greater understanding of how Plant Plus could be effectively used in the management of macropods.

Macropus rufogriseus banksianus were utilised as a test species for these trials due to their abundance in NSW, their frequent occurrence as roadkill in NSW and their availability for captive study (Section 1.3). While there are many similarities in the ecology and biology of *M. rufogriseus banksianus* and other species of macropod, it is not possible to assume that the repellent effects of Plant Plus demonstrated with captive *M. rufogriseus banksianus* will be apparent for other species. The five most significant macropod roadkill victims in south-eastern Australia include *M. giganteus*, *M. fuliginosis*, *M. rufus* (red kangaroo), *W. bicolor* and *M. rufogriseus banksianus* (Coulson, 1997). Repellents can affect non-target species in numerous ways including attracting some species (Bullard *et al.*, 1978; Muller-Schwarze, 1990; Apfelbach *et al.*, 2005). Before Plant Plus can be used as a mitigative measure for macropod-vehicle collisions, further trials are required to assess the effectiveness of Plant Plus with a range of macropod species and determine potential effects for non-target species.

Many different types of odours (predator, irritating, offensive: natural or synthetic) have been trialled as repellents with varying degrees of success (see Albone, 1990; Muller-Schwarze, 1990; Lindgren *et al.*, 1995; Mason *et al.*, 2001; Apfelbach *et al.*, 2005 and Appendix C). When trialled under field conditions, repellents often show reduced efficacy or different results than expected when compared to analogous captive trials (Nolte, 2003; Apfelbach *et al.*, 2005). Due to the wide range of results reported (often for the same repellent), it is apparent that further research is required in two areas: the potential utility of odours as widespread and multi-species management tools (including identifying potential environmental impacts); and detailed identification of the properties of the most successful repellents (including Plant Plus).

There have been only a limited number of field trials investigating animal repellents in Australia and results have been generally disappointing (see Coleman *et al.*, 2006). However, a clear need for the use of alternative non-lethal control methods exists for both browsing management (Bulinski & McArthur, 2003; Witt *et al.*, 2003; Walsh & Wardlaw, 2005;

Coleman *et al.*, 2006) and for the management of wildlife in other situations including road management (Donaldson & Bennett, 2004; Magnus *et al.*, 2004).

The occurrence of macropod-vehicle collisions is a significant problem in New South Wales and Australia. Macropod-vehicle collisions can cause property damage and human injury. Insurance costs, aesthetics and animal welfare are also adversely affected by macropod-vehicle collisions. Several roadkill mitigation techniques exist (Section 1.2.1.2), however, no one technique is fully effective and the effectiveness of many mitigation methods are unknown. An integrated approach to the problem of macropod-vehicle collisions is required that utilises many mitigation strategies. New and innovative approaches, including the use of repellents, are required to compliment the suite of existing mitigation measures.

Overall, Plant Plus has shown promise as a repellent, significantly reducing feeding and movements of *M. rufogriseus banksianus*. There was only minor habituation detected after six weeks and Plant Plus can remain effective in the field for up to 10 weeks. Further study is required to determine if Plant Plus is effective for other species of large macropod, especially utilising field studies. Further investigations into the properties of Plant Plus are required to determine if Plant Plus is suitable for broad-scale deployment as a management tool including as a roadkill abatement technique. Suitable application methods and potential environmental impacts must also be assessed.

7.2 Recommendations

A number of factors requiring further investigation were highlighted by the research conducted and reported in this thesis. Further research into potential repellents for use in Australia is required and should include an assessment of a broad array of compounds both individually and in combination. This research should include an assessment of DMDT, IPMS and chicken eggs (including the recently released Pestat synthetic fermented egg spray). The identification of scenarios where repellents may be of most benefit should also proceed.

Research to determine the utility of Plant Plus as a suitable repellent to reduce macropod-vehicle collisions in New South Wales should proceed. Both captive and field trials are required to reveal the full potential of Plant Plus as a roadkill mitigation option and include the following.

Captive Studies:

- *Determination of the response mechanism of M. rufogriseus banksianus to Plant Plus*. An understanding of how and why *M. rufogriseus banksianus* respond to Plant Plus will result in an improved knowledge on how to best use Plant Plus as a management tool. It may also reveal alternative avenues for management and lead to an increased understanding of the contexts in which Plant Plus can be used.

- *Clarification of the strength of response exhibited to Plant Plus and identification of changes that may occur over time (including identification of product-related longevity).* The strength of response to a repellent determines its effectiveness in a variety of circumstances. Plant Plus was very effective when alternative food sources were available, but was not quite as effective when no alternatives were provided (Chapter 3). Determination of the strength of the response will lead to a greater understanding of where and when Plant Plus may be effective. While Plant Plus was observed to invoke an aversive response after ten weeks, limitations in methods prevented an evaluation of the change in response over time. Focus should be placed on determining a more accurate assessment of product related longevity and the relationship between time and strength of product.

- *Quantification of the variation in responses to Plant Plus by macropods.* One group of animals tested during the habituation trials (Chapter 4) responded quite differently to all other groups. An understanding of the natural variation in responses is required to fully assess the potential of Plant Plus as a management technique. The effects of social facilitation, gender discrepancies and any other influencing factors on response require detailed investigation.

- *Elucidation of the dose dependant response relationship of Plant Plus and effects on rates of habituation and extinction.* The strength of response and rate of habituation of response are related to the concentration of Plant Plus deployed. Elucidation of the optimal concentration of Plant Plus that invokes the desired response (including minimal habituation and/or extinction) will lead to improved management outcomes and cost effectiveness.

- *Further identification of the repellent properties of Plant Plus.* It was demonstrated that Plant Plus was an effective area repellent. However, elucidation of the exact properties (distance of effect) will enable efficient deployment and lead to a greater understanding of the context in which Plant Plus may be effective.

- *Trials with other large macropods.* The ability of Plant Plus to invoke a response in other large macropods needs to be determined before it can be utilised as a roadkill mitigation measure. All trials conducted with Plant Plus and subjects from the *Macropus* genus have so far been successful and may be indicative of a genera wide response. Testing of this hypothesis is required, prioritising the species often associated with macropod-vehicle collisions (*M. giganteus*, *M. rufus*, *M. fuliginosus, M. robustus* and *M. agilis*).

- *Trials with selected non-target organisms.* Several repellents have been identified as attractants for non-target species, especially predators. Trials to determine if Plant Plus is an attractant should proceed to ensure that usage does not result in increased densities of non-target animals (e.g. predators) and/or incidences of animal-vehicle collisions.

- *Identification of application methods for repellents, suitable for broad-scale and long-term use.* Plant Plus was effective after 10 weeks and may be effective continuously in the field if topically applied quarterly. However, methods of application need to be identified that maximise effects and reduce quantities of Plant Plus used. Microencapsulation and slow-release devices are particularly worthy of investigation.

Field Studies:

- *Identification of the strength of response by free-ranging macropods to Plant Plus.* The response of macropods to Plant Plus in field conditions has not been determined. The response of animals in the field does not always resemble responses observed in captivity. It is of high importance that the responses of macropods under field conditions be assessed and compared to the responses observed in captive trials. This must occur before the development of Plant Plus as an effective management technique.

- *Assessment of the ability of Plant Plus to reduce macropod densities in road easements and to reduce macropod-vehicle collisions.* The premise of Plant Plus as a roadkill mitigative strategy relies on reducing macropod densities in road easements and/or increasing avoidance behaviours of animals in the easement. These factors have not been investigated and this underlying assumption of the mitigative technique needs to be fully researched as a priority.

- *Investigate repellent application regimes and interactions with other management strategies.* While captive studies investigating application regimes are important, an understanding of how Plant Plus can add to an integrated management plan is also important. Generally, management techniques are more effective when integrated and applied in a plan involving several techniques. However, an understanding of how each technique interacts is important. Research should determine if existing management techniques could be improved by the incorporation of Plant Plus. Such techniques could include fencing or provision of diversionary/alternative resources.

- *A cost benefit analysis and an assessment of potential environmental impacts of Plant Plus.* The environmental impact of widespread application and a cost benefit analysis are necessary before wide-scale use of Plant Plus as a management tool. Additionally, further investigation into macropod-vehicle collisions is required to identify how and where repellents could provide the most benefit.

This research has identified Plant Plus as an effective repellent for *M. rufogriseus banksianus* with potential for use in the management of this species due to its effectiveness in reducing visitation to, and food consumption in, treated areas. These effects were achieved over prolonged periods without the target species habituating to the repellent. These encouraging findings warrant further investigation to assess if Plant Plus can be utilised to mitigate vehicle-macropod collisions in New South Wales.

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Appendix A

Permits and Approvals

File No . AW2002/015
Trim File No. 02/1926

ANIMAL RESEARCH AUTHORITY

Issued by the
DIRECTOR-GENERAL
OF NSW AGRICULTURE

Principal Investigator: **Mr Craig Gibson**
Australian Catholic University
PO Box 968
NORTH SYDNEY NSW 2059

Associate Investigator: **Dr Scott Wilson**
Dr Vaughan Monamy

are authorised
to conduct the following research

A Preliminary Assessment of Chemical Repellents in the Management of Vehicle-Marsupial Collisions in rural NSW

Location: **University of New South Wales field stationn COWAN**

as approved by and in accordance with the
ANIMAL CARE AND ETHICS COMMITTEE OF THE DIRECTOR-GENERAL OF NSW AGRICULTURE

being animal research carried out in accordance with the Code of Practice, for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

*This authority remains in force until **17 June 2003** unless suspended, cancelled or surrendered.*

(Signature of authorised person)

ROSS BURTON
MANAGER, ANIMAL WELFARE UNIT

14 August 2002

File No. AW2002/015
Trim File No. 02/1926

ANIMAL CARE AND ETHICS COMMITTEE OF THE DIRECTOR-GENERAL OF NSW AGRICULTURE CERTIFICATE OF APPROVAL

Mr Craig Gibson
Australian Catholic University
PO Box 968
NORTH SYDNEY NSW 2059

is approved
to conduct the following research

A Preliminary Assessment of Chemical Repellents in the Management of Vehicle-Marsupial Collisions in rural NSW

as approved by and in accordance with the
ANIMAL CARE AND ETHICS COMMITTEE OF THE DIRECTOR-GENERAL OF NSW AGRICULTURE

being animal research carried out in accordance with the Code of Practice, for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

This approval remains in force until ***17 June 2005*** *unless suspended, cancelled or surrendered.*

Amanda Paul

(Signature of authorised person)

AMANDA PAUL
EXECUTIVE OFFICER

14 August 2002

Trim File No. 02/1926

ANIMAL RESEARCH AUTHORITY

Issued by the
DIRECTOR-GENERAL
OF NSW AGRICULTURE

Principal Investigator: **Mr Craig Gibson**
Australian Catholic University
PO Box 968
NORTH SYDNEY NSW 2059

Associate Investigators: **Dr Scott Wilson**
Dr Vaughan Monamy

are authorised
to conduct the following research

Management of vehicle-marsupial collisions using repellents:
Longevity and Habituation trials

Location: **University of New South Wales field station Cowan**

as approved by and in accordance with the
ANIMAL CARE AND ETHICS COMMITTEE OF THE DIRECTOR-GENERAL OF NSW AGRICULTURE

being animal research carried out in accordance with the Code of Practice, for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

*This authority remains in force until **25 August 2004** unless suspended, cancelled or surrendered.*

(Signature of authorised person)

ROSS BURTON
MANAGER, ANIMAL WELFARE UNIT

25 September 2003

Trim File No. 02/1926

ANIMAL CARE AND ETHICS COMMITTEE OF THE DIRECTOR-GENERAL OF NSW AGRICULTURE CERTIFICATE OF APPROVAL

Mr Craig Gibson
Australian Catholic University
PO Box 968
NORTH SYDNEY NSW 2059

is approved
to conduct the following research

Management of vehicle-marsupial collisions using repellents: Longevity and Habituation trials

as approved by and in accordance with the
ANIMAL CARE AND ETHICS COMMITTEE OF THE DIRECTOR-GENERAL OF NSW AGRICULTURE

being animal research carried out in accordance with the Code of Practice, for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

*This approval remains in force until **25 August 2004** unless suspended, cancelled or surrendered.*

Amanda Paul

(Signature of authorised person)

AMANDA PAUL
EXECUTIVE OFFICER

25 September 2003

Trim File No. 02/1926

ANIMAL RESEARCH AUTHORITY

Issued by the
DIRECTOR-GENERAL OF
NSW DEPARTMENT OF PRIMARY INDUSTRIES

Principal Investigator: Mr Craig Gibson
Australian Catholic University
Centre for Environmental Restoration and Stewardship
PO Box 968
NORTH SYDNEY NSW 2059

Associate Investigators: Dr Scott Wilson
Dr Vaughan Monamy

are authorised
to conduct the following research

A Preliminary Assessment of Chemical Repellents in the Management of Vehicle-Marsupial Collisions in rural NSW

Location: **Tomago Sandbeds at Medowie and Salt Ash New South Wales**

as approved by and in accordance with the
ANIMAL CARE AND ETHICS COMMITTEE OF THE DIRECTOR-GENERAL OF NSW DEPARTMENT OF PRIMARY INDUSTRIES

being animal research carried out in accordance with the Code of Practice, for a recognised research purpose and in connection with animals (other than exempt animals) that have been obtained from the holder of an animal suppliers licence.

*This authority remains in force from **1 June 2005 – 30 September 2005** unless suspended, cancelled or surrendered.*

(Signature of authorised person)

ROSS BURTON
MANAGER, ANIMAL WELFARE UNIT

5 August 2005

THE UNIVERSITY OF
NEW SOUTH WALES

Authority to Conduct Animal Research Project

ANIMAL CARE
AND ETHICS COMMITTEE
(ACEC)

ACE Number:	**02/90**
Title:	A premiminary assessment of chemical repellents in the management of vehicle-marsupial collisions in rural NSW
Start/Expiry Dates	14 August 2002 to 13 August 2005
Name of the Researcher:	Mr Craig Gibson
Address:	Centre for Environmental Restoration and Stewardship Australian Catholic University, North Sydney
Contact Phone Number:	9739 2547 (04121730420)
Email Address:	gibsoncp@hotmail.com

Authorised Personnel A/Prof Gail Crossley, Dr Scott Wilson, Dr Vaughan Monamy, Mr Bruce McNamara, Dr David Croft

Species/Strain	**Year 1**	**TOTAL**
Red-necked wallabies *(Macropus rufogriseus)*	10	10

Conditions of Approval Particular to this Project : NIL

Conditions of Approval Applicable to All Projects

1. ***All cages holding animals for this project should be labelled with its ACE Number, Expiry Date, Chief Investigator, and Contact Phone Number, as listed above.***
2. ***Modifications to this project and the addition of new personnel must have prior written approval of the ACEC. Please send requests to (ethics.sec@unsw.edu.au).***
3. ***All projects are subject to annual review by the ACEC.***
4. ***Please have this letter of authority available during site inspection by ACEC members.***
5. ***Please distribute copies of this letter of authority to the other authorised personnel listed above (copies enclosed).***
6. ***In the event of an unforeseen adverse incident (e.g. unexpected deaths), the NHMRC Code of Practice (3.1.11) requires that investigators promptly notify the ACEC (m.wright@unsw.edu.au).***
7. ***If a project involves surgery, the researchers are required to monitor animals daily for at least first week post operatively.***

Authorised on behalf of the Vice-Chancellor on 14 August 2002

A/Professor Michael Perry, Presiding Member, ACEC

UNSW SYDNEY NSW 2052
AUSTRALIA
Telephone: +61 (2) 9385 4234
Facsimile: +61 (2) 9385 6648
Email: ethics.sec@unsw.edu.au

THE UNIVERSITY OF
NEW SOUTH WALES

Authority to Conduct Animal Research Project

ANIMAL CARE
AND ETHICS COMMITTEE
(ACEC)

ACE Number:	**03/68**
Title:	Management of vehicle-marsupial collisions using repellents: Longevity and Habituation trials
Start/Expiry Dates	8 October 2003 to 7 October 2006
Name of the Researcher:	Mr Craig Gibson
Address:	Centre for Environmental Restoration & Stewardship, Australian Catholic University
Contact Phone Number:	9739 2547
Email Address:	gibsoncp@hotmail.com

Authorised Personnel **Dr Scott Wilson, Dr Vaughan Monamy, Ms Cath Dunstan**

Species/Strain	Year 1	Year 2	Year 3	TOTAL
Red–necked Wallabies (Macropus rufogriseus)	………………. Not Applicable ………..			

Conditions of Approval Particular to this Project Nil

Conditions of Approval Applicable to All Projects

1. ***All cages holding animals for this project should be labelled with its ACE Number, Expiry Date, Chief Investigator, and Contact Phone Number, as listed above.***
2. ***Modifications to this project and the addition of new personnel must have prior written approval of the ACEC. Please send requests to (ethics.sec@unsw.edu.au).***
3. ***All projects are subject to annual review by the ACEC.***
4. ***Please have this letter of authority available during site inspection by ACEC members.***
5. ***Please distribute copies of this letter of authority to the other authorised personnel listed above (copies enclosed).***
6. ***In the event of an unforeseen adverse incident (e.g. unexpected deaths), the NHMRC Code of Practice (3.1.11) requires that investigators promptly notify the ACEC (m.wright@unsw.edu.au).***
7. ***If a project involves surgery, the researchers are required to monitor animals daily for at least first week post operatively.***

Authorised on behalf of the Vice-Chancellor on 8 October 2003

A/Professor Michael Perry, Presiding Member, ACEC

UNSW SYDNEY NSW 2052
AUSTRALIA
Telephone: +61 (2) 9385 4234
Facsimile: +61 (2) 9385 6648
Email: ethics.sec@unsw.edu.au

Scientific Investigation Licence
Section 120
National Parks and Wildlife Act, 1974

Dr Scott WILSON
Australian Catholic University
Sch. of Arts & Science
NORTH SYDNEY NSW 2059

Licence Number: A3238 Expires: 31-May-2003

Project: Preliminary assessment of chemical repellants in the management of roadkill

Authorised Officer: Date: 29/5/02

The licensee is authorised to :
harm/study Wallabia bicolor

in the following areas : -
non-Service areas

in conjunction with the project, subject to the conditions contained in the application form and the following conditions :

Signature of Licensee : Date : 3 · 6 · 02

Scientific Investigation Licence
Section 120
National Parks and Wildlife Act, 1974

Mr Craig Phillip GIBSON
Australian Catholic Univeristy
PO Box 968
SYDNEY NSW 2059

Licence Number: B2392 Expires: 31-May-2003

Project: Preliminary assessment of chemical repellants in the management of roadkill

Authorised Officer: Schumacher Date: 27/6/02

The licensee is authorised to :
harm/study Wallabia bicolor

in the following areas : -
non-Service areas

in conjunction with the project, subject to the conditions contained in the application form and the following conditions :

Signature of Licensee : Date : 12.8.02

COPY

Scientific Licence
Section 132C, NPW Act 1974

Mr Craig Phillip GIBSON
Australian Catholic University, MacKillop Campus
40 Edward Street
NORTH SYDNEY NSW 2059

Licence Number: S10625 Expires: 31-May-2004

Project: Management of vehicle-marsupial collisions using repellents

Authorised Officer: Date: 30 OCT 2003

The licensee is authorised to :
harm/study Macropus rufogriseus banksianus

in the following areas : -
non-Service areas

in conjunction with the project, subject to the conditions contained in the application form and the following conditions :
Vaughan Monamy and Scott Wilson are also authorised under this licence

Signature of Licensee : Date :

Scientific Licence
Clause 22, NPW Regulation 2002
and Section 132C, NPW Act 1974

Mr Craig GIBSON
Australian Catholic University
40 Edward St
North Sydney NSW 2059

Licence Number: S11617 Expires: 30-Jun-2006

Project: Management of Macropods using repellents-a field trial

Authorised Officer: Date: 29 JUN 2005

The licensee is authorised to :
Harm, conduct research using repellents on, macropods, conduct work on DEC managed estate

in the following areas : -
SCA lands with SCA approval

in conjunction with the project, subject to the conditions contained in the application form and the following conditions :
Fauna to be managed as per a current ACEC approval. Also authorised Vaughan Monamy, Scott Wislon. SCA approval must be gained prior to work on SCA managed lands.

Signature of Licensee : Date : 5/7/05

HUNTER WATER

caring for our community and the environment

HUNTER WATER CORPORATION
PO BOX 5171 HRMC NSW 2310
426-432 KING STREET NEWCASTLE WEST
TEL: 1300 657 657 ABN: 46 228 513 446
www.hunterwater.com.au

AUTHORITY No. 794

AUTHORITY TO ENTER

Approval is hereby given

to: Craig Gibson

of: Australian Catholic University
PO Box 968
NORTH SYDNEY NSW 2059

Telephone: 0421 730 420 (W) 9739 2547 (H) 9568 5818

to enter upon: Tomago Sandbeds

from: 1 October 2004 to 20 February 2005 plus two days a later date.

for the purpose of: Conducting a research project on red-neck wallabies.

Approval to enter upon the land is given subject to the following conditions:-

1. Access will be permitted only to the land marked by yellow tint on the attached plan and only for the purpose of conducting your research project on the approved dates.
2. That you enter the land at your own risk and indemnify Hunter Water Corporation (HWC) against all claims and actions arising out of your entry upon and use of the land and that you indemnify HWC against any damage or injury to its property or personnel arising from your entry upon and use of the land.
3. That you accept responsibility for all parties involved in your activities including but not limited to volunteers and observers.
4. That you exercise proper precautions to prevent any damage whatsoever to HWC property or facilities on the land. You will not obstruct or hinder HWC or any other authorised party from undertaking work on the land.
5. Access to and across the land will be confined to recognised access roads, tracks and firebreaks as shown on the attached plan.
6. No waste, litter or other substance will be brought onto or left on the land.
7. No plant, shrub, tree or other vegetation will be disturbed, damaged or removed from the land.
8. You must notify the Corporation's Ranger Mr Ian Graham on phone number 49 799 847 or mobile 0419 604895 at least 24 hours prior to entering the land to confirm your entry and use arrangements.
9. The land is within a Special Area under the Hunter Water (Special Areas) Regulations 2003. The objectives of the Regulations are to protect the quality and quantity of water in the Special Area for the supply of potable water to the Lower Hunter Region. Special Areas Regulations

Page 2 **AUTHORITY No.**

must be observed at all times.

10. The land is to remain in a clean, tidy and undisturbed condition. Toilet wastes shall be disposed of outside of the HWC's catchment area or otherwise at an approved disposal facility.

11. Williamtown RAAF Base, the Air Weapons Range and the Parachute Dropping Zone areas are controlled by the Commonwealth of Australia and access into these areas is subject to the directions and authority of the RAAF Security Police. Access to these areas may be denied.

12. HWC gives no warranty or undertaking to make or keep the land secure and will accept no responsibility for loss or damage to any material, equipment or other thing brought onto or stored on the land. All items stored on the land will be removed to the satisfaction of HWC.

13. Keys for HWC gates may be obtained from Francine O'Brien, on (02) 4979 9498. A fee may be charged for entry into secured areas. A key application form is attached.

14. Any instruction or direction given by a HWC Ranger or any other authorised officer will be observed and complied with.

15. The Authority to Enter does not extend to other lands owned by HWC and access onto other lands is not permitted under the Authority to Enter.

16. HWC reserves the right to suspend entry onto the land at any time and without notice in the event of urgent works being required or if your activities are deemed to be causing an adverse impact on HWC land or operations or infrastructure.

17. The Authority to Enter may be withdrawn by the HWC at any time without notice.

18. The Authority to Enter is valid only for the approved period from 1 October 2004 to 28 February 2005.

19. Prior to your entry onto the Tomago Sandbeds you are required to have a Site Induction by the Corporation's Ranger Mr Ian Graham and complete the attached "Site Specific OH&S Induction Sheet. This will need to be completed prior to the submission of your application for a key to enter the site. Please contact Ian on phone number 49 799 847 or mobile 0419 604895 to arrange for a time and date for the induction.

Please report any incident or problem immediately by telephoning 1300 657 000.

This Authority must be kept in your possession and must be produced upon demand by any officer of the Corporation or authorised person.

Bob Gumb
Real Estate Drafter
Property Management
27 September 2004

HUNTER WATER

caring for our community and the environment

HUNTER WATER CORPORATION
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426-432 KING STREET NEWCASTLE WEST
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www.hunterwater.com.au

AUTHORITY No. 794

AUTHORITY TO ENTER

Approval is hereby given

to:	Craig Gibson
of:	Australian Catholic University PO Box 968 NORTH SYDNEY NSW 2059
Telephone:	0421 730 420 (W) 9739 2547 (H) 9568 5818
to enter upon:	Tomago Sandbeds
from:	21 February 2005 to 30 April 2005.
for the purpose of:	Conducting a research project on red-neck wallabies.

Approval to enter upon the land is given subject to the following conditions:-

1. Access will be permitted only to the two areas of land marked by yellow tint on the attached plan and only for the purpose of conducting your research project on the approved dates.

2. That you enter the land at your own risk and indemnify Hunter Water Corporation (HWC) against all claims and actions arising out of your entry upon and use of the land and that you indemnify HWC against any damage or injury to its property or personnel arising from your entry upon and use of the land.

3. That you accept responsibility for all parties involved in your activities including but not limited to volunteers and observers.

4. That you exercise proper precautions to prevent any damage whatsoever to HWC property or facilities on the land. You will not obstruct or hinder HWC or any other authorised party from undertaking work on the land.

5. Access to and across the land will be confined to recognised access roads, tracks and firebreaks as shown on the attached plan.

6. No waste, litter or other substance will be brought onto or left on the land.

7. No plant, shrub, tree or other vegetation will be disturbed, damaged or removed from the land.

8. You must notify the Corporation's Ranger Mr Ian Graham on phone number 49 799 847 or mobile 0419 604895 at least 24 hours prior to entering the land to confirm your entry and use arrangements.

9. The land is within a Special Area under the Hunter Water (Special Areas) Regulations 2003. The objectives of the Regulations are to protect the quality and quantity of water in the Special Area for the supply of potable water to the Lower Hunter Region. Special Areas Regulations must be observed at all times.

Page 2 **AUTHORITY No.**

10. The land is to remain in a clean, tidy and undisturbed condition. Toilet wastes shall be disposed of outside of the HWC's catchment area or otherwise at an approved disposal facility.

11. Williamtown RAAF Base, the Air Weapons Range and the Parachute Dropping Zone areas are controlled by the Commonwealth of Australia and access into these areas is subject to the directions and authority of the RAAF Security Police. Access to these areas may be denied.

12. HWC gives no warranty or undertaking to make or keep the land secure and will accept no responsibility for loss or damage to any material, equipment or other thing brought onto or stored on the land. All items stored on the land will be removed to the satisfaction of HWC.

13. Keys for HWC gates may be obtained from Francine O'Brien, on (02) 4979 9498. A fee may be charged for entry into secured areas. A key application form is attached.

14. Any instruction or direction given by a HWC Ranger or any other authorised officer will be observed and complied with.

15. The Authority to Enter does not extend to other lands owned by HWC and access onto other lands is not permitted under the Authority to Enter.

16. HWC reserves the right to suspend entry onto the land at any time and without notice in the event of urgent works being required or if your activities are deemed to be causing an adverse impact on HWC land or operations or infrastructure.

17. The Authority to Enter may be withdrawn by the HWC at any time without notice.

18. The Authority to Enter is valid only for the approved period from 1 October 2004 to 28 February 2005.

Please report any incident or problem immediately by telephoning 1300 657 000.

This Authority must be kept in your possession and must be produced upon demand by any officer of the Corporation or authorised person.

Bob Gumb
Real Estate Drafter
Property Management
15 February 2005

HUNTER WATER
caring for our community and the environment

HUNTER WATER CORPORATION
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www.hunterwater.com.au

AUTHORITY No. 794

AUTHORITY TO ENTER

Approval is hereby given

to: Craig Gibson

of: Australian Catholic University
PO Box 968
NORTH SYDNEY NSW 2059

Telephone: 0421 730 420 (W) 9739 2547 (H) 9568 5818

to enter upon: Tomago Sandbeds

from: 1 May 2005 to 31 July 2005.

for the purpose of: Conducting a research project on red-neck wallabies.

Approval to enter upon the land is given subject to the following conditions:-

1. Access will be permitted only to the two areas of land marked by yellow tint on the attached plan and only for the purpose of conducting your research project on the approved dates.
2. That you enter the land at your own risk and indemnify Hunter Water Corporation (HWC) against all claims and actions arising out of your entry upon and use of the land and that you indemnify HWC against any damage or injury to its property or personnel arising from your entry upon and use of the land.
3. That you accept responsibility for all parties involved in your activities including but not limited to volunteers and observers.
4. That you exercise proper precautions to prevent any damage whatsoever to HWC property or facilities on the land. You will not obstruct or hinder HWC or any other authorised party from undertaking work on the land.
5. Access to and across the land will be confined to recognised access roads, tracks and firebreaks as shown on the attached plan.
6. No waste, litter or other substance will be brought onto or left on the land.
7. No plant, shrub, tree or other vegetation will be disturbed, damaged or removed from the land.
8. You must notify the Corporation's Ranger Mr Ian Graham on phone number 49 799 847 or mobile 0419 604895 at least 24 hours prior to entering the land to confirm your entry and use arrangements.
9. The land is within a Special Area under the Hunter Water (Special Areas) Regulations 2003. The objectives of the Regulations are to protect the quality and quantity of water in the Special Area for the supply of potable water to the Lower Hunter Region. Special Areas Regulations must be observed at all times.

Page 2 **AUTHORITY No.**

10. The land is to remain in a clean, tidy and undisturbed condition. Toilet wastes shall be disposed of outside of the HWC's catchment area or otherwise at an approved disposal facility.

11. Williamtown RAAF Base, the Air Weapons Range and the Parachute Dropping Zone areas are controlled by the Commonwealth of Australia and access into these areas is subject to the directions and authority of the RAAF Security Police. Access to these areas may be denied.

12. HWC gives no warranty or undertaking to make or keep the land secure and will accept no responsibility for loss or damage to any material, equipment or other thing brought onto or stored on the land. All items stored on the land will be removed to the satisfaction of HWC.

13. Keys for HWC gates may be obtained from Francine O'Brien, on (02) 4979 9498. A fee may be charged for entry into secured areas. A key application form is attached.

14. Any instruction or direction given by a HWC Ranger or any other authorised officer will be observed and complied with.

15. The Authority to Enter does not extend to other lands owned by HWC and access onto other lands is not permitted under the Authority to Enter.

16. HWC reserves the right to suspend entry onto the land at any time and without notice in the event of urgent works being required or if your activities are deemed to be causing an adverse impact on HWC land or operations or infrastructure.

17. The Authority to Enter may be withdrawn by the HWC at any time without notice.

18. The Authority to Enter is valid only for the approved period from 1 October 2004 to 28 February 2005.

Please report any incident or problem immediately by telephoning 1300 657 000.

This Authority must be kept in your possession and must be produced upon demand by any officer of the Corporation or authorised person.

Bob Gumb
Real Estate Drafter
Property Management
3 May 2005

Australian Pesticides & Veterinary Medicines Authority

PERMIT TO ALLOW THE CONDUCT OF SMALL-SCALE TRIALS WITH AGVET CHEMICALS

Permit Number – PER 7250

This permit, is issued under section 114 of the Agvet Codes, to allow those persons stipulated below to conduct small-scale trials in any jurisdiction, as defined in the conditions of use, with agricultural or veterinary chemical active constituents or products, whether such active constituents or products are approved or registered or not. The permit also allows those persons stipulated below to possess any active constituent or chemical product described below for the purposes described under this permit.

CONDITIONS OF USE

1. Persons who can conduct trials under this permit:
All persons who are trained or experienced in the handling and use of agricultural or veterinary chemicals and who handle and use agricultural or veterinary chemicals as part of their normal duties in their employment for an organisation for which they are conducting a trial;

2. Jurisdictions in which this permit applies
All jurisdictions.

3. Products/Actives that can be used under this permit
Any active constituent or chemical product, except:

- one which is or contains a genetically modified organism; or
- veterinary biologicals used outside the confines of a research facility; or
- any active constituent or chemical product used in a trial where the trial is conducted in a jurisdiction where that active constituent or chemical product is proscribed by legislation or
- any active constituent or chemical product whose use has been prohibited under the Agricultural and Veterinary Chemicals (Administration) Regulations 2004.

4. ***Purpose/Situation***
For the conduct of 'Small-Scale Trials' that, for the purposes of this permit, are defined as:

(i) screening tests, laboratory assessment and other research conducted within the confines of a research facility. (A research facility includes research station, research laboratory, research glasshouse, veterinary surgery or hospital, university or similar institution); or

(ii) trials conducted to generate data relating to efficacy, residues, crop or animal safety or other scientific information outside the confines of a research facility where the size of the trial annually does not exceed the following:

a. a total of 5 hectares nationally , with a maximum of 1 hectare in any one jurisdiction in the case of any food and/or fibre field crop or
b. a total of 500 plants nationally with a maximum of 100 plants in any one jurisdiction for plants other than those in a food and/or fibre field crop; or
c. a total of 100 cattle, pigs, or deer; 1000 sheep or goats; or 2000 poultry; or 100 non-food animal species; or
d. patch trials of antifouling paint products where the area treated on each vessel does not exceed 10 square metres and no more than 2 vessels in any one jurisdiction or a total of 10 vessels nationally are treated; or
e. raft panel trials using antifouling paints where the total national area of treated panel does not exceed 100 square metres with a maximum of 10 square metres treated at any one site; or
f. 5 cubic metres of timber or timber products in any one jurisdiction, or a total of 25 cubic metres nationally; or
g. any other situation where the total area treated nationally is not greater than 10 sq metres; or
h. fumigation trials conducted under the Australian Standard for General Fumigation Procedures (AS 2476-1981) where the total national area treated does not exceed 400 cubic metres with a maximum of 160 cubic metres to be treated per jurisdiction and no individual treatment site exceeds 40 cubic metres.

ADDITIONAL CONDITIONS

5. Do not dispose of any produce from plants or animals treated during a trial that can result in direct or indirect consumption of this produce by humans for a period of 12 months from application of the agricultural or veterinary chemical.

6. Do not dispose of or allow the use of any treated commodity or item that will result in direct or indirect exposure of humans to the agricultural or veterinary chemical used in the trial.

7. Persons handling/applying the agricultural or veterinary chemical for the purposes of conducting the trial must wear appropriate personal protective equipment to minimise their exposure to the agricultural or veterinary chemical via the eyes, skin, nose or mouth.

8. All trials involving animals must comply with conditions laid down in animal welfare legislation or guidelines, which are applicable in the jurisdiction where trials are conducted.

9. The organisation or individual for which the trial is being conducted must maintain detailed records listing:

a. the date the trial is conducted;

b. for trials conducted within the confines of a research facility, the name and address of the research facility; for trials conducted outside the confines of a research facility, the jurisdiction and specific location within each jurisdiction that the trials are conducted;

c. the trial details, including plants, animals or items treated, the pest controlled or reason for treating, the rates and frequency of application;

d. the active constituents or chemical products used plus the total amounts used;

e. the method of disposal of produce from treated plants or animals; and

f. the names of the persons conducting or controlling the trials.

10. The organisation or individual for which the trial is being conducted must maintain the records described in ***9 a.- f.*** above for each trial for a period of not less than 2 years from the date of commencing each trial and such records must be made available to the APVMA upon their request.

This permit remains in force until it is cancelled.

DATED: 7 June 2004

Eva Bennet – Jenkins

……………………………………..
(signature of delegate)

Appendix B

Review of Roadkill in Australia

Appendix B

A review of scientific literature regarding vehicle-animal collisions in Australia. The table is sorted by location. A brief summary is included.

Location of Study	Focus of Paper/Comments	Citation
-	A review of ecological effects of roads. A good summary of some specific examples of roadkill and roadkill mitigation in Australia. Other ecological effects of roads were discussed.	(Andrews, 1990)
-	A review of wildlife conservation in relation to roads and roadsides. This included an overview of many aspects of road ecology with an excellent review of "Road systems and the mortality of wildlife".	(Bennett, 1991)
-	A review of the influence that roads may play in the dispersal of predators in Australia and the role roads may play in facilitating predation. This paper highlighted the need for research into the impact of exotic predators on native small mammals under various stages of road construction and road densities. The fragmentation caused by roads may also facilitate exotic predators in restricting population size of small mammals.	(May & Norton, 1996)
-	This outlined the opinion of what research was required in field of road ecology. It was suggested that roadkill is the largest problem associated with roads. Small-scale studies were suggested on the spatial and temporal distribution of roadkill as well as mitigative measures.	(Ramp, 2004)
Australian Capital Territory	Summarises the extent of the macropod roadkill problem in the ACT. A conclusion of the report was that no mitigative technique currently available is likely to be fully effective, even when mitigation techniques are used in combination. Deterrent devices were suggested as an avenue of further research, however, the	(Committee, 1997)

Location of Study	Focus of Paper/Comments	Citation
	cost-effectiveness of deterrent methods were questioned. Driver awareness and driver behaviour modification were suggested as the only viable mitigative measures to reduce macropod roadkill in the ACT.	
Australian Capital Territory	A study of *Macropus giganteus* roadkill in urban areas of the ACT. Data was collected over 2.5 years and included 1068 macropod roadkill. Lunar phase, season and gender were significant factors in the occurrence of *M. giganteus* roadkill. Interaction effects between these factors were also noted along with an interaction effect between macropod age and season.	(Lintermans & Cunningham, 1997)
Australian Capital Territory	Examined the feasibility of a trial to test swareflex wildlife reflectors. The costs of road-based field trials were found to be prohibitive if trial design was to be effective. This was based on statistical analysis of data collected for macropod roadkill at hotspots in the ACT (high variability was noted). It was recommended that work should instead focus on the underlying assumptions of the reflectors (that the light is detected by macropods, effects on behaviour etc.).	(Lintermans, 1997)
New South Wales	This paper focused on the movement of 3 *Rattus sp*. and 1 *Melomys sp*. in pine plantation, including in relation to roads. Road crossings by the species studied were inversely related to road width. Even unused and/or overgrown roads restricted or ceased mammal movement.	(Barnett *et al.*, 1978)
New South Wales	A study of vertebrate roadkill trends from data collected by animal rescue volunteers. The densities of roadkill varied enormously between sites. Over 85% of roadkill noted were native animals.	(Cooper, 1998)
New South Wales	Mammal use of railway underpass. Underpasses with established vegetation at either end were utilised successfully by native small mammals. Newly constructed underpasses and underpasses devoid of vegetation at entrances were predominately used by feral predators.	(Hunt *et al.*, 1987)

Location of Study	Focus of Paper/Comments	Citation
New South Wales	A study of causal factors of kangaroo-vehicle collisions in western NSW in drought conditions and following drought. The roadkill rate for the 4 kangaroo species investigated was much higher in drought when compared to non-drought periods. The majority of roadkill victims were young individuals (2 years of age). Curves in the road and the presence of stock races increased the likelihood of roadkill. The frequency of kangaroo roadkill was a function of density of animals in the road easement, night time traffic volume, low rainfall, vegetation cover and the relative greenness of vegetation along the road relative to surrounding areas. It was postulated that the selective mortality source of kangaroos affects population structure.	(Lee *et al.*, 2004)
New South Wales Royal National Park, Sydney.	A study of roadkill in the Royal National Park and a survey of driver attitudes. 112 vertebrate roadkill specimens were collected over 144 survey days spanning a 22 km stretch of road. More roadkill were observed when traffic volume was low and during periods of low rainfall. More roadkill was found near drainage culverts and in areas where there was lots of vegetation on the road verge. A survey of drivers revealed that willingness to modify driving behaviour to reduce roadkill increased with age.	(Morrissey, 2004)
New South Wales	Assessment of underpass use by fauna. Underpasses were used by a wide range of fauna. Fauna used underpasses of all sizes studied (1.5 m -10 m diameter). The study did not include many underpasses but found that the largest underpass assessed was used by the most diverse array of fauna. However, the smallest underpass was used most frequently.	(Norman *et al.*, 1998)
New South Wales	Progress report on road ecology research. Trials of roadside reflectors in a simulated environment indicated a possible partial effect for two macropod species. Further evaluation was required. Preliminary results on the use of predator odours with two small macropods also suggested further research was	(Ramp & Croft, 2002)

Location of Study	Focus of Paper/Comments	Citation
	required.	
New South Wales	Opinion on mammal use of road, rail and power easements and suggested mitigative measures for roads. It was suggested that roads have more detrimental effects on mammal species than either rail or power easements. Some engineered finishings to major roads were suggested (e.g. road tunnels instead of deep cuttings).	(Robinson, 1976)
New South Wales	The efficacy of fauna underpasses when used in conjunction with exclusionary fencing was assessed. Surveys of fauna in habitat surrounding roads and the monitoring of underpass usage and roadkill, revealed that the underpasses in conjunction with exclusionary fencing were effective at reducing roadkill while facilitating movement across the road easement. Underpasses were effective for a range of fauna species, however, underpasses were not effective for frogs. A large number of frogs were found dead on the roads.	(Taylor & Goldingay, 2003)
New South Wales	A study of roadkill in Northern New South Wales. 529 roadkill were described. The problems associated with survey methods were discussed. It was determined that further study of the local populations of *Isoodon macrourus* was needed to determine if road mortality was significantly affecting demographics. *Isoodon macrourus* were the most frequent mammalian roadkill. Landscape attributes were measured and some were found to be related to the presence of roadkill of particular species or groups of species.	(Taylor & Goldingay, 2004)
New South Wales	An account of roadkill on a 65 km stretch of road in New South Wales. Data was collected on 234 days over a period of one year. It was also noted that roadkill often disappear from the road surface within one day. The roadkill observed included: 155 birds; 100 mammals; and 44 reptiles. A species list was provided but no analysis was conducted.	(Thomas, 1988)

Location of Study	Focus of Paper/Comments	Citation
New South Wales & Australian Capital Territory, Canberra to Murrumbateman	A survey of bird roadkill. Two years of survey revealed seasonal patterns in road deaths for several species. *Gymnorhina tibicen* (magpie) and *Cacatua roseicapilla* (galah) were the most frequently observed roadkill species. Roadkill peaked between September and November and also in Autumn. The lowest numbers of roadkill were observed in winter. No threatened species were found killed on roads.	(Lepschi, 1992)
New South Wales & Victoria	A short investigation of vertebrate roadkill. More birds than mammals were killed in spring. In autumn, the number of birds and mammals killed were almost equal, with slightly more mammals found as roadkill.	(Disney & Fullagar, 1978)
New South Wales & Australian Capital Territory	Roadkill surveys were conducted monthly over two years on a 301 km stretch of road. A total of 1675 roadkill were collected. Season, landscape and road characteristics were all suggested as factors in the location and number of roadkill. Birds accounted for 66% of the roadkill observed and mammals 29%. Some difficulties in survey methods were introduced and it was noted that carcasses rapidly disappeared from the road surface.	(Vestjens, 1973)
New South Wales, Sydney.	Examined the mortality of *Tiliqua scincoids* in an urban environment. Roadkill of *T. scinoids* were found to be seasonal and peak periods were correlated with peak activity periods. Roadkill accounted for 12% of the mortalities noted.	(Koenig *et al.*, 2002)
Queensland	*Phascolarctos cinerus* (koala) mortality in a metropolitan shire was studied in conjuction with a speed-zone trial designed to reduce mortality. Most roadkill of *P. cinerus* were young healthy males. Enforceable differential speed restrictions did not slow vehicle traffic and did not reduce roadkill. Many factors contributed to the location and density of *P. cinerus* roadkill.	(Dique *et al.*, 2003)

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Queensland	The designs of four large underpasses to allow for the movements of native fauna were discussed with a proposal of methods to test the effectiveness of the underpasses. It included a review of underpass design theory and usage in Australia and a description of furniture to encourage particular species using the underpasses and to allow predator avoidance strategies.	(Goosem *et al.*, 2001)
Queensland	Edge effects were detected with some road verges providing habitat for exotic species. Native species also showed a gradient with *Melomys cervinipes* being found near the roads in greater numbers than expected with *Rattus sp.* increasing with distance from the road.	(Goosem, 2000)
Queensland	A narrow unsealed road was found to be a significant barrier for some small mammals. There was a differing effect between species, and also seasons. The differences between species were attributed to size, mobility and behaviour. Suggested reasons for the seasonal differences were dispersion of juveniles and increased activity during breeding seasons. Culverts were found to be more effective when vegetation was present at the openings.	(Goosem, 2001)
Queensland	A recovery plan for *Petrogale Persephone* (Proserpine rock wallaby). Indicated success of trials using roadside reflectors in reducing road mortality of *P. persephone*.	(Nolan & Johnson, 2001)
Queensland	Analysis of *Wallabia bicolor* roadkill on North Stradbroke Island. Roadkill of *W. bicolor* were significantly correlated with the occurrence of faecal pellet counts in disturbed areas along the roadside. This was interpreted by suggesting that *W. bicolor* were attracted to disturbed areas along the roadside and were vulnerable to collisions in these areas. Lunar cycle did not appear to be a factor in the collision rate, however traffic density was. There were 127 roadkill analysed for this study.	(Osawa, 1989)
Queensland, Wet	Roads inhibited movement of two small mammal species, but a larger species was not inhibited. Roads	(Goosem, 2002)

Location of Study	Focus of Paper/Comments	Citation
tropics	affected community structure, however, traffic had no detectable impact. A flaw was noted with the traffic study as differences in traffic were determined per day, but it is possible the two traffic levels were similar during the active period for the animals (night).	
Queensland, Marburg	Use of roadside remnants by birds. Highlighted the diversity of birds found in some roadside remnants and the need for conserving roadside remnants.	(Leach & Recher, 1993)
Queensland, Wet tropics	Three of four small mammal species were noted to have reduced movement across a narrow bitumen road. Of the species studied, *Uromys caudimaculatus* had the highset road-crossing rate.	(Burnett, 1992)
Queensland, Wet tropics	Easements were found to be a significant barrier for small rainforest dwelling mammals. Gullies where vegetation existed allowed the movement of animals, however, where a large gap in the canopy was located, small mammals were inhibited in movement.	(Goosem & Marsh, 1997)
South Australia, Coorong region	Investigation of the impact of off road vehicles (ORV) on *Charadrius rubricollis* (hooded plovers). It was concluded that the reproductive rate of *C. rubricollis* is potentially reduced by ORV usage on beaches in South Australia. Up to 81% of nests on beaches were potentially destroyed by being run-over during the nesting period.	(Buick & Paton, 1989)
South Australia, Coorong region	Investigated the environmental effects of off road vehicles on the Coorong dune system, with particular emphasis on geomorphic impacts. The impact on plants and animals from accelerated dune erosion was discussed. The spread of exotic species, fragmentation and noise impacts were also mentioned. Implications for the management of these areas were the focus of the paper.	(Gilbertson, 1983)
Tasmania	An analysis of *Perameles gunnii* (eastern barred bandicoot) habitat requirements utilising roadkill data. Traffic volume was the major determinant of roadkill of *P. gunnii*. Rainfall and soil depth were also	(Driessen *et al.*, 1996)

Location of Study	Focus of Paper/Comments	Citation
	strongly associated with the occurrence of roadkill. It was suggested that rainfall and increased soil depth are favoured by *P. gunnii* and may explain the relationship with roadkill.	
Tasmania	Following the improvement of a section of road, local populations of *Dasyurus viverrinus* and *Sacrophilus laniarius* crashed. An increase in the number of roadkill at the same time was observed. Following the implementation of mitigation techniques (slow points/chicanes, rumple bars and warning signs, education campaigns, wildlife reflectors and "wildlife ramps" over ditches and embankments) the amount of roadkill decreased and the population of *D. viverrinus* recovered. Some indications of recovery for *S. laniarius* were also noted.	(Jones, 2000)
Tasmania	A review and assessment of roadkill mitigation techniques, specifically for use in Tasmania. Wildlife signage, escape routes, table drain (ditch) management, platypus crossings, underpasses and odour repellents were identified as measures likely or potentially likely to reduce wildlife roadkill. Ultrasonic whistles, wildlife reflectors and lighting were assessed as having little value in roadkill mitigation for Tasmania. Further research, towards a greater understanding of roadkill events and sites, and wildlife behaviour in reaction to oncoming traffic was recommended.	(Magnus *et al.*, 2004)
Tasmania	A roadkill mitigation review designed for use by road and land managers (e.g. local government and state departments). The kit consisted of five sections and extended from Magnus *et al.* (2004). The kit contained easy to use flow charts as decision-making tools and highlighted relevant legislation regarding the implementation of mitigative measures for roadkill.	(Magnus, 2006)
Tasmania Launceston	Over a period of one year, 23 *T. vulpecula* were tracked in an urban environment. At least nine of these individuals died as a result of colliding with cars and two others were non-fatally injured but required	(Stratham & Stratham, 1997)

Location of Study	Focus of Paper/Comments	Citation
	veterinary treatment. A further 5 individuals could not be located at the end of the study and it is possible that they were also victims of roadkill. This study highlighted the high mortality rates of *T. vulpecula* due to roads and traffic in Launceston.	
Tasmania	Speculation about the reasons for *Ornithorhynchus anatinus* roadkill. It is noted the *O. anatinus* cross roads when creeks and streams flow under the road. It is suggested that *O. anatinus* leave streams to cross roads because of the heavy modification of the waterway. This results in a greater than expected mortality rate. This issue is further discussed by Magnus *et al.* (2004).	(Tyson, 1980)
Tasmania, Huon valley	A study of *Perameles gunnii* demographics. Preliminary data suggested that roadkill of *P. gunnii* are a feasible and reliable source of population data for *P. gunnii*. The implications of using such data both temporally and spatially were discussed.	(Mallick *et al.*, 1998)
Tasmania, Deloraine	More roadkilll was found on an elevated section of road than a ground level road. *Gallinula mortierii* (Tasmanian native hen) and several birds of prey were more vulnerable on the elevated section of road. Platypuses were noted as vulnerable as it is hypothesised that they do not use modified environments (culverts) under roads.	(Taylor & Mooney, 1991)
Victoria	Reviews the benefits of roadside verges for wildlife and detrimental affects of roadway. In an issue devoted to roads and roadsides with focus on rural and regional areas of Victoria.	(Anderson, 1977)
Victoria	Roadkill mitigation using ultrasonic whistles designed for attachment to vehicles. This study focused on macropods. Ultrasonic emission was detectable at 50 metres. However, there was no indication that macropods could detect the emission and there was no evidence that the emission altered the behaviour of captive animals. Devices attached to vehicles did not reduce roadkill.	(Bender, 2001)

Location of Study	Focus of Paper/Comments	Citation
Victoria	Further investigated the use of ultra sonic devices. It was revealed that the devices did not alter behaviour in two macropod species in either captive or field-based trials.	(Bender, 2003)
Victoria	An investigation of habitat corridors, remnant forest patches and roadside verges as conduits for mammal dispersal. Roadside vegetation was used by mammals in a number of ways. Use of the roadside vegetation was not limited to generalist or common species. 18 species (out of 23 known from the area) used the roadside vegetation. Roadsides were important links between remnants. Wildlife conservation was dependant on preserving habitat continuity in the area studied. Roadside reserves were suggested.	(Bennett, 1988)
Victoria	It was suggested that roadside verges provided habitat suitable for dispersion of small mammals. Vegetation of roadsides should be preserved.	(Bennett, 1990)
Victoria	An investigation of macropod roadkill. Roadkill was seasonal. Roadkill was a selective mortality factor with more males being affected than females. Lunar cycle may influence occurrence of roadkill. Landscape was a factor in determining where roadkill will occur. Warning signs appeared ineffective at reducing collisions.	(Coulson, 1982)
Victoria	Discussed difficulties in collecting accurate roadkill data, differential risks between species, differential risks within species (e.g. between sexes), dangers to humans, uses of roadkill in monitoring populations and the ineffectiveness of roadside warning signs in reducing the amount of roadkill.	(Coulson, 1985)
Victoria	It was noted that the roadkill of two species of macropod was greater during drought. The seasonal frequency of macropod roadkill was inversely related to the previous season's rainfall. Roadkill was predominately males, with almost half of the roadkill juvenile specimens.	(Coulson, 1989)
Victoria	In 5 species of macropod, a significant bias towards males being killed on roads was found. *Macropus*	(Coulson, 1997)

Location of Study	Focus of Paper/Comments	Citation
	rufus was the only species were no bias was detected.	
Victoria	A good review of Australian studies of roadkill and road ecology. No new information included. However, an emphasis was placed on the management of roads in National Parks with some recommendations suggested.	(Donaldson & Bennett, 2004)
Victoria	Some studies have suggested that feral predators use roads for dispersion and that the distribution of these predators is related to roads. However, during this study there was no detectable influence of roads determining the location of predators.	(Lindenmayer *et al.*, 1994)
Victoria	An article referring to a work in progress that was later published (Mansergh & Scotts, 1989). The vulnerable *Burramys parvus* was photographed utilising a furnished tunnel under a road. Male *B. parvus* segregate from females and a road was preventing the segregation. This decreased the birth and survival rates until the tunnel was built.	(Steer, 1987)
Victoria	Outline of Victorian plans to conserve remnant vegetation in road easements. This paper also reviewed some specific roadkill issues and mitigative measures. It is highlighted that roadkill impacts on different species in various ways and that mitigation methods need to be targeted and specific. An overview of roadkill issues and mitigation techniques were discussed for *Psephotus haematogaster* (bluebonnet), *Polytelis anthopelus* (regent parrot), *Pomatostmus temporalis* (grey-crowned babbler), *Kakotoe roseicapilla* (galah), *Kakotoe galerita* (sulphur-crested cockatoo), *Eudyptula minor* (fairy penguin), *Dasyurus viverrinus* (eastern quoll), *Perameles gunnii* (eastern barred bandicoot), *Wallabia bicolor* (swamp wallaby), *Potorous tridactylus* (long-nosed potoroo), *Petrogale persephone* (proserpine rock wallaby), *Phascolarctos cinereus* (koala), *Burramys parvus* (mountain pygmy possum) and *Macropus*	(Straker, 1998)

Location of Study	Focus of Paper/Comments	Citation
	rufus (red kangaroo).	
Victoria, Kiamal	Several events where large numbers of the endangered *Polytelis anthopeplus monarchoides* (regents parrot) have been killed eating grain spilled on road by trucks were reported. The roadside was graded to reduce the availability of grain on the roadside and the numbers of roadkill decreased. It was also suggested that following grain spills, heavy watering could also reduce roadkill by germinating the seeds and removing them as a food source for birds.	(Anon, 1980)
Victoria, Mt Hotham	Two furnished (imitating boulder/scree) underpasses beneath a road bisecting the breeding grounds of *Burramys parvus* (mountain pygmy possum) restored connectivity and stabilised the population. The disruptive scale of roads for the life cycle of *B. parvus* was discussed and recommendations were made regarding underpasses and other wildlife.	(Mansergh & Scotts, 1989)
Victoria, Phillip Island	Investigated the causes of mortality for *Eudyptula minor* (fairy penguin) in coastal regions. Post mortem examination of specimens revealed that trauma related to vehicle collisions was a major source of mortality for *E. minor*.	(Harrigan, 1992)
Western Australia	Examined the problem of bird roadkill in the wheatbelt. It was noted that a high proportion of juveniles were killed. Large numbers of roadkill occurred where creeks and roads intersected. *Gymnorhina tibicen* (Australian magpie), *Grallina cyanoleuca* (magpielark) and *Platycercus icterotis* (western rosella) were frequent victims.	(Brown *et al.*, 1986)
Western Australia	Investigated the use of road verges by birds in a highly fragmented habitat (central wheatbelt of WA). Diversity of all bird species was inversely related with distance from road verge in the fragmented habitat. A similar relationship was observed between the numbers of birds dependant on native vegetation and	(Fortin & Arnold, 1997)

Location of Study	**Focus of Paper/Comments**	**Citation**
	distance form road verge. Road verges were important habitat for many bird species.	
Western Australia, Wheatbelt region	The high abundance and diversity of reptilian roadkill was reported for a major highway north of Perth. The abundance of reptilian roadkill in nature reserves was also discussed and several management strategies used in nature reserves in WA were highlighted as being detrimental for fauna and likely to increase roadkill. Alternatives to these management strategies were suggested.	(Bush *et al.*, 1991)
Western Australia, Garden Island	One hundred and eighty-seven roadkill *Macropus* eugenii (tammar wallaby) were collected over eight months in 2004 on Garden Island. Older animals were more frequent victims of roadkill and overall no sex bias was detected. Roadkill of *M. eugenii* on Garden Island may be reducing the population size. Ongoing research is investigating the impacts of roadkill on the Garden Island population and also mitigative measures.	(Chambers & Bencini, 2005)

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Appendix C

Review of Repellent Research

Appendix C

A partial review of research regarding repellents for use with mammals (Predator odour section adapted from Apfelbach *et al.* (2005)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
Predator (Whole animal)	Ferret *Mustela putorius* Least weasel *Mustela nivalis* Racoon dog *Nyctereutes procyonides* Red fox *Vulpes vulpes* Stoat *Mustela erminea* Stone marten *Martes foina*	Bank vole *Clethrionomys glareolus*	Behavior	yes	(Jedrzejewski *et al.*, 1993)
	Tawny owl *Strix aluca*	Bank vole *Clethrionomys glareolus*	Behavior	no	(Jedrzejewski *et al.*, 1993)
	Least weasel *Mustela nivalis*	Bank vole *Clethrionomys glareouls*	Space use	yes	(Jedrzejewski & Jedrzejewska, 1990)
	Least weasel *Mustela nivalis*	Bank vole *Clethrionomys glareolus* Field vole *Microtus agrestis*	Space use	yes	(Borowski & Owadowska, 2001)
	Least weasel *Mustela nivalis*	Field vole *Microtus agrestis*	Space use	yes	(Korpimaki *et al.*, 1996)
	Least weasel *Mustela nivalis*	Field vole *Microtus agrestis*	Feeding rate	yes	(Koivisto & Pusenius, 2003)
	Stoat *Mustela erminea*	Bank vole *Clethrionomys glareolus*	Reproduction	yes	(Ylonen & Ronkainen, 1994)
	Stoat *Mustela erminea*	Deer mouse *Peromyscus maniculatus*	Analgesia	yes	(Kavaliers, 1990)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Stoat *Mustela erminea*	Meadow vole *Microtus pennsylvanicus*	Behavior	yes	(Parsons & Bondrupnielsen, 1996)
	Stoat *Mustela erminea*	Root vole *Microtus oeconomus*	Space use	yes	(Borowski, 1998)
	Least weasel *Mustela nivalis*	Bank vole *Clethrionomys glareolus*	Feeding rate	no	(Sundell *et al.*, 2004)
Predator (Faeces)	African lion *Panthera leo*	European rabbit *Oryctolagus cuniculus*	Feeding rate	yes	(Boag & Mlotkiewicz, 1994)
	African lion *Panthera leo* Bengal tiger *Panthera tigris tigris* Brown bear *Ursus arctos* Siberian tiger *Panthera tigris altaica*	Alpine goat *Capra hircus*	Feeding rate	yes	(Weldon *et al.*, 1993)
	Cougar *Felis concolor*	Alpine goat *Capra hircus*	Feeding rate	no	(Weldon *et al.*, 1993)
	African lion *Panthera leo* Bengal tiger *Panthera tigris tigris* Cougar *Felis concolor* Coyote *Canis latrans* Snow leopard *Panthera uncia*	Black tailed deer *Odocoileus hemionus columbianus*	Feeding rate	yes	(Muller-Schwarze, 1972)
	Black bear *Ursus americanus* Coyote *Canis latrans* Dog *Canis familiarus* Lynx *Lynx canadensis* River otter *Lutra canadensis* Wolf *Canis lupus*	North American beaver *Castor canadensis*	Feeding rate	yes	(Englehart & Muller-Schwarze, 1995)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Bobcat *Lynx rufus* Cougar *Felis concolor* Coyote *Canis latrans* Lynx *Lynx canadensis* Wolf *Canis lupus*	Black tailed deer *Odocoileus hemionus columbianus* Snowshoe hare *Lepus americanus*	Feeding rate	yes	(Sullivan *et al.*, 1985a, 1985b)
	Brown bear *Ursus arctos* Lynx *Lynx lynx* Red fox *Vulpes vulpes* River otter *Lutra lutra* Wolf *Canis lupus*	Eurasian beaver *Castor fiber*	Feeding rate	yes	(Rosell & Czech, 2000)
	Dog *Canis familiarus*	Eurasian beaver *Castor fiber*	Feeding rate	no	(Rosell & Czech, 2000)
	Carpet python *Morelia spilota variegata* Dingo *Canis familiarus dingo* Red fox *Vulpes vulpes* Spotted-tailed quoll *Dasyurus maculatus* Tasmanian devil *Sarcophilus harrisii*	Bush rat *Rattus fuscipes* Giant white-tailed rat *Uromys caudimaculatus* Fawn-footed melomys *Melomys cervinipes*	Behavior	yes	(Hayes *et al.*, 2006)
	Cat *Felis cattus*	House mouse *Mus domesticus*	Behavior	yes	(Berton *et al.*, 1998)
	Cat *Felis cattus* Mink *Mustela vison* Red fox *Vulpes vulpes*	Field vole *Microtus agrestis*	Physiology (body mass)	yes	(Carlsen *et al.*, 1999)
	Cat *Felis cattus* Mongoose *Herpestes auropunctatus*	Brown rat *Rattus norvegicus*	Behavior	yes	(Bramley *et al.*, 2000)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Cat *Felis cattus* Red fox *Vulpes vulpes* Western quoll *Dasyurus geoffroii*	House mouse *Mus domesticus*	Space use, Trapping rate	yes	(Dickman, 1992)
	Cat *Felis cattus* Shrew *Sorex sp.*	House mouse *Mus domesticus*	Trapping rate	yes	(Drickamer *et al.*, 1992)
	Dog *Canis familiarus*	House mouse *Mus domesticus*	Trapping rate	no	(Drickamer *et al.*, 1992)
	Cougar *Felis concolor* Coyote *Canis latrans* Red fox *Vulpes vulpes*	Cattle *Bos taurus* Sheep *Ovis ares*	Feeding rate	yes	(Pfister *et al.*, 1990)
	Cougar *Felis concolor* Wolf *Canis lupus*	Wapiti *Cervus elephas canadiensis*	Physiology (heart rate, O_2 consumption rate)	yes	(Chabot *et al.*, 1996)
	Dog *Canis familiarus* Wolf *Canis lupus*	Sheep *Ovis ares*	Feeding rate	yes	(Arnould *et al.*, 1998)
	European badger *Meles meles*	European hedgehog *Erinaceus europaeus*	Behavior, Trapping rate	yes	(Ward *et al.*, 1997)
	European badger *Meles meles* Red fox *Vulpes vulpes*	Bank vole *Clethrionomys glareolus* Field vole *Microtus agrestis* Wood mouse *Apodemus sylvaticus*	Trapping rate	yes	(Dickman & Doncaster, 1984)
	Red fox *Vulpes vulpes*	Shrew *Sorex araneus*	Trapping rate	no	(Dickman & Doncaster, 1984)
	Jaguar *Panthera onca* Jaguarandi *Herpailurus yaguaroundi* Margay *Leopardus (Felis) wiedii*	Red-bellied tamarin *Saguinus labiatus*	Behavior	yes	(Caine & Weldon, 1989)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Least weasel *Mustela nivalis*	Field vole *Microtus agrestis*	Space use, Feeding rate, Behavior	yes	(Bolbroe *et al.*, 2000)
	Least weasel *Mustela nivalis*	Field vole *Microtus agrestis*	Feeding rate	yes	(Koivisto & Pusenius, 2003)
	Mongoose *Herpestes auropunctatus*	Black rat *Rattus rattus*	Behavior, Feeding rate	yes	(Burwash *et al.*, 1998)
	Mongoose *Herpestes auropunctatus*	Black rat *Rattus rattus* Polynesian rat *Rattus exulans*	Behavior, Trapping rate	yes	(Tobin *et al.*, 1995)
	Red fox *Vulpes vulpes*	House mouse *Mus domesticus*	Feeding rate	yes	(Coulston *et al.*, 1993)
	Red fox *Vulpes vulpes*	Meadow vole *Microtus pennsylvanicus* Montane vole *Microtus montanus*	Feeding rate, Trapping rate	yes	(Sullivan *et al.*, 1988a)
	Red fox *Vulpes vulpes*	Meadow vole *Microtus pennsylvanicus*	Space use	yes	(Perrot-Sinal *et al.*, 1999)
	Red fox *Vulpes vulpes*	Orkney vole *Microtus arvalis*	Feeding rate, Trapping rate	yes	(Calder & Gorman, 1991)
	Sand boa *Eryx jaculus*	Common spiny mouse *Acomys cahrinus*	Analgesia	yes	(Carere *et al.*, 1999)
	Stoat *Mustela erminea*	Root vole *Microtus oeconomus*	Space use	yes	(Borowski, 1998)
	Western quoll *Dasyurus geoffroii*	Ash-grey mouse *Pseudomys albocinereus* Yellow-footed antechinus *Antechinus flavipes*	Trapping rate	yes	(Dickman, 1993)
	Bobcat *Lynx rufa*	Meadow vole *microtus pennsylvanicus*	Feeding rate	no	(Pusenius & Ostfeld, 2002)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Blandford's fox *Vulpes cana*	Common spiny mouse *Acomys cahrinus* Golden spiny mouse *Acomys russatus*	Feeding rate	no	(Jones & Dayan, 2000)
	Cat *Felis cattus*	Ash-grey mouse *Pseudomys albocinereus* Yellow-footed antechinus *Antechinus flavipes*	Trapping rate	no	(Dickman, 1993)
	Dingo *Canis familiarus dingo* Kodiak bear *Ursus arctos* Red fox *Vulpes vulpes*	Red-necked pademelon *Thylogale thetis* Tammar wallaby *Macropus eugenii*	Feeding rate	no	(Blumstein *et al.*, 2002)
	Dog *Canis familiarus*	Brown antechinus *Antechinus stuartii* Bush rat *Rattus fuscipes*	Trapping rate	no	(Banks *et al.*, 2003)
	Mink *Mustela vison*	Gray-tailed vole *Microtus canicaudus*	Space use	no	(Wolff & Davisborn, 1997)
	Red fox *Vulpes vulpes*	Brown rat *Rattus norvegicus*	Behavior	no	(McGregor *et al.*, 2002)
	Red fox *Vulpes vulpes*	Bush rat *Rattus fuscipes*	Trapping rate	no	(Banks, 1998)
	Red fox *Vulpes vulpes*	House mouse *Mus domesticus*	Feeding rate, Population dynamics	no	(Banks & Powell, 2004)
	Stoat *Mustela erminea*	Meadow vole *Microtus pennsylvanicus*	Trapping rate	no	(Parsons & Bondrupnielsen, 1996)
	Wolverine *Gulo gulo*	Snowshoe hare *Lepus americanus*	Feeding rate	no	(Sullivan, 1986)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
Predator (Urine)	African lion *Panthera leo*	North American beaver *Castor canadensis*	Feeding rate	yes	(Englehart & Muller-Schwarze, 1995)
	Bobcat *Lynx rufa*	Meadow vole *Microtus pennsylvanicus* Woodchuck *Marmota monax*	Feeding rate	yes	(Swihart, 1991)
	Bobcat *Lynx rufa* Coyote *Canis latrans* Dog *Canis familiarus* Mink *Mustela vison*	Mountain beaver *Aplodontia rufa*	Feeding rate	yes	(Epple *et al.*, 1993; Hubbard *et al.*, 2004)
	Bobcat *Lynx rufus* Coyote *Canis latrans* Fox *Vulpes vulpes* Lynx *Lynx canadensis* Wolf *Canis lupus* Wolverine *Gulo gulo*	Black-tailed deer *Odocoileus hemionus columbianus* Snowshoe hare *Lepus americanus*	Feeding rate	yes	(Sullivan *et al.*, 1985b, 1985a)
	Dog *Canis familiarus*	Snowshoe hare *Lepus americanus*	Feeding rate	no	(Sullivan *et al.*, 1985b, 1985a)
	Cat *Felis cattus*	Brown rat *Rattus norvegicus*	Behavior	yes	(Bramley *et al.*, 2000)
	Cat *Felis cattus*	Campbell's hamster *Phodopus campbelli*	Endocrinology, Reproduction	yes	(Vasilieva *et al.*, 2000)
	Coyote *Canis latrans*	Deer mouse *Peromyscus numiculatus* Guinea pig *Cavia porcellus* House mouse *Mus domesticus* Mountain beaver *Aplodontia rufa*	Feeding rate	yes	(Nolte *et al.*, 1994)
	Coyote *Canis latrans*	Woodchuck *Marmota monax*	Feeding rate	yes	(Bean *et al.*, 1995)

Origin of repellent	**Odour source**	**Target species**	**Tested Effects**	**Repellence detected**	**Citation**
	Coyote *Canis latrans*	Cow elk *Cervus elapus nelsoni*	Feeding rate	yes	(Andelt *et al.*, 1992)
	Coyote *Canis latrans*	Mule deer *Odocoileus hemionus*	Feeding rate	Weak response	(Andelt *et al.*, 1991)
	Dog *Canis familiarus*	Swamp wallaby *Wallabia bicolor*	Feeding rate	yes	(Montague *et al.*, 1990)
	Red fox *Vulpes vulpes*	Swamp wallaby *Wallabia bicolor*	Feeding rate	no	(Montague *et al.*, 1990)
	Dog *Canis familiarus* (synthetic)	Parma wallaby *Macropus parma*	Behaviour	yes	(Ramp *et al.*, 2005)
	Dog *Canis familiarus* (synthetic)	Red-necked pademelon *Thylogale thetis*	Behaviour	no	(Ramp *et al.*, 2005)
	Ferret *Mustela putorius furo*	Mouse *Mus musculus*	Behaviour	yes	(Roberts *et al.*, 2001)
	Gray fox *Urocyon cinereoargenteus*	Kangaroo rat *Dipodomys merriami*	Feeding rate	yes	(Herman & Valone, 2000)
	Human *Homo sapiens* Racoon *Procyon lotor* Red fox *Vulpes vulpes*	Grey squirrel *Sciurus canadensis*	Feeding rate	yes	(Rosell, 2001)
	Mongoose *Herpestes auropunctatus*	Black rat *Rattus rattus* Polynesian rat *Rattus exulans*	Behavior, Trapping rate	yes	(Tobin *et al.*, 1995)
	Red fox *Vulpes vulpes*	Meadow vole *Microtus pennsylvanicus* Montane vole *Microtus montanus*	Feeding rate	yes	(Sullivan *et al.*, 1988a)
	Red fox *Vulpes vulpes*	Snowshoe hare *Lepus americanus*	Feeding rate	yes	(Sullivan & Crump, 1986)
	Stoat *Mustela erminea*	Root vole *Microtus oeconomus*	Space use	yes	(Borowski, 1998)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Tiger *Panthera tigris*	Field vole *Microtus agrestis*	Trapping rate	yes	(Stoddart, 1982a; Stoddart, 1982b)
	Wolverine *Gulo gulo*	Snowshoe hare *Lepus americanus*	Feeding rate	yes	(Sullivan, 1986)
	Bobcat *Lynx rufa* Coyote *Canis latrans* Ocelot *Leopardus pardalis* Red fox *Vulpes vulpes*	Oldfield mice *Peromyscus polionotus*	Feeding rate	no	(Orrock *et al.*, 2004)
	Bobcat *Lynx rufa* Coyote *Canis latrans* Red fox *Vulpes vulpes*	Meadow vole *microtus pennsylvanicus*	Feeding rate	no	(Pusenius & Ostfeld, 2002)
	Caracal *Felis caracal* Leopard *Panthera pardus*	Cape grysbok *Raphicerus melanotis* Grey duiker *Sylvicapra gimmia* Striped field mouse *Rhabdomys pumillio* Vlei rat *Otomys irroratus*	Feeding rate, Trapping rate	no	(Novellie *et al.*, 1982)
	Cat *Felis cattus*	Black rat *Rattus rattus* Polynesian rat *Rattus exulans*	Behavior, Feeding rate	no	(Bramley & Waas, 2001)
	Dog *canis familiarus* Human *Homo sapiens*	Red-necked pademelon *Thylogale thetis* Tammar wallaby *Macropus eugenii*	Feeding rate	no	(Blumstein *et al.*, 2002)
	Jaguar *Panthera onca*	Field vole *Microtus agrestis*	Trapping rate	no	(Stoddart, 1980)
	Mink *Mustela vison*	Gray-tailed vole *Microtus canicaudus*	Space use	no	(Wolff & Davisborn, 1997)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Mink *Mustela vison*	Prairie vole *Microtus ochrogaster* Woodland vole *Microtus pinetorum*	Behaviour	no	(Wolff, 2004)
Predator (Anal gland secretion)	Ferret *Mustela putorius* Mink *Mustela vison* Stoat *Mustela erminea*	Pocket gopher *Thomomys talipoides*	Trapping rate	yes	(Sullivan *et al.*, 1988b; Sullivan *et al.*, 1990)
	Least weasel *Mustela nivalis*	Field vole *Microtus agrestis*	Trapping rate	yes	(Stoddart, 1976, , 1980)
	Least weasel *Mustela nivalis*	Wood mouse *Apodemus sylvaticus*	Trapping rate	no	(Stoddart, 1976, , 1980)
	Mink *Mustela vison*	Bank vole *Clethrionomys glareolus* European rabbit *Oryctolagus cuniculus* Field mouse *Apodemus sylvaticus*	Feeding rate, Trapping rate	yes	(Robinson, 1990)
	Mink *Mustela vison*	Mountain beaver *Aplodontia rufa*	Feeding rate	yes	(Epple *et al.*, 1993)
	Mink *Mustela vison* Stoat *Mustela erminea*	Snowshoe hare *Lepus americanus*	Feeding rate	yes	(Sullivan & Crump, 1984; Sullivan *et al.*, 1985b)
	Ferret *Mustela putorius* Least weasel *Mustela nivalis*	Snowshoe hare *Lepus americanus*	Feeding rate	no	(Sullivan & Crump, 1984; Sullivan *et al.*, 1985b)
	Stoat *Mustela erminea*	Field voile *Microtus agrestis* Orkney vole *Microtus arvalis*	Trapping rate	yes	(Gorman, 1984)
	Stoat *Mustela erminea*	Wood mouse *Apodemus sylvaticus*	Trapping rate	no	(Gorman, 1984)
	Stoat *Mustela erminea*	House mouse *Mus domesticus*	Feeding rate	yes	(Coulston *et al.*, 1993)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Stoat *Mustela erminea*	Meadow vole *Microtus pennsylvanicus* Montane vole *Microtus montanus*	Feeding rate, Trapping rate	yes	(Sullivan *et al.*, 1988a)
	Siberian weasel *Mustela sibiricus*	Golden hamster *Mesocricetus auratus* Rat-like hamster *Cricetulus triton*	Endocrinology, Reproduction	yes	(Zhang *et al.*, 2003)
	Synthetic mustelid odor	Brushtail possum *Trichosurus vulpecula* European rabbit *Oryctolagus cuniculus*	Feeding rate	yes	(Morgan & Woolhouse, 1995; Woolhouse & Morgan, 1995)
	Synthetic mustelid odor	Black rat *Rattus rattus* Polynesian rat *Rattus exulans*	Behavior, Feeding rate	no	(Bramley & Waas, 2001)
	Synthetic mustelid odor	Meadow vole *Microtus pennsylvanicus* Montane vole *Microtus montanus*	Feeding rate	no	(Sullivan *et al.*, 2004)
	Brown rat *Rattus norvegicus* (cage litter) Mink *Mustela vison* (cage litter)	Water vole *Arvicola terrestris*	Behavior	yes	(Barreto & Macdonald, 1999)
Predator (other odour - bedding, fur, cloth etc)	Cat *Felis cattus* (cloth)	Brown rat *Rattus norvegicus*	Behavior	yes	(Blanchard *et al.*, 1990; File *et al.*, 1993; Li *et al.*, 2004)
	Cat *Felis cattus* (collar)	Brown rat *Rattus norvegicus*	Behavior	yes	(Dielenberg *et al.*, 1999)
	Cat *Felis cattus* (ball of fur)	Brown rat *Rattus norvegicus*	Behavior	yes	(Vazdarjanova *et al.*, 2001)
	Coyote *Canis latrans* (bedding)	Snowshoe hare *Lepus americanus*	Feeding rate	yes	(Sullivan *et al.*, 1985b)

Origin of repellent	**Odour source**	**Target species**	**Tested Effects**	**Repellence detected**	**Citation**
	Ferret *Mustela putois*	Brown rat *Rattus norvegicus*	Behavior, Endocrinology	yes	(Masini *et al.*, 2005)
	Least weasel *Mustela nivalis* (bedding)	Grey-sided voles *Clethrionomys rufocanus*	Reproduction	yes	(Fuelling & Halle, 2004)
	Least weasel *Mustela nivalis* (cage wash)	Root vole *Microtus oeconomus*	Trapping rate	yes	(Borowski, 2002)
	Stoat *Mustela erminea* (cage wash)	Root vole *Microtus oeconomus*	Feeding Behavior	yes	(Borowski, 1998)
	Least weasel *Mustela nivalis* (bedding)	Bank vole *Clethrionomys glareolus*	Feeding rate	no	(Sundell *et al.*, 2004)
Non-predator based	Chicken Eggs Ropel Thiram	Elk *Cervus elapus*	Feeding rate	no	(Andelt *et al.*, 1992; Baker *et al.*, 1999)
	MGK Big Game Repellent (Deer Away) Hinder Hot Sauce Animal Repellent	Elk *Cervus elapus*	Feeding rate	yes	(Andelt *et al.*, 1992; Baker *et al.*, 1999)
	MGK Big Game Repellent Chicken Eggs Hot Sauce	Mule deer *Odocoileus hemionus*	Feeding rate	Weak response	(Andelt *et al.*, 1991; Andelt *et al.*, 1994)
	Ropel Thiram Bars of soap Habernero peppers Tabasco sauce Ani-spray	Mule deer *Odocoileus hemionus*	Feeding rate	no	(Andelt *et al.*, 1991; Andelt *et al.*, 1994)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	MGK Big Game Repellent Hinder	White-tailed deer *Odocoileus virginianus*	Feeding rate	yes	(Conover, 1984)
	Thiram Human hair Magic circle Miller Hot Sauce	White-tailed deer *Odocoileus virginianus*	Feeding rate	Weak response	(Conover, 1984)
	Chicken egg/acrylic mix	Brush-tail possum *Trichosurus vulpecular*	Feeding rate	yes	(Eason & Hickling, 1992)
	Burnt possum *Trichosurus vulpecula* pelt Burnt *Trichosurus vulpecula* testes	Brush-tail possum *Trichosurus vulpecular*	Feeding rate	no	(Eason & Hickling, 1992)
	Cinnamamide	House mice *Mus musculus*	Feeding rate	Yes	(Gurney *et al*., 1996)
	Cinnamamide	Wood mice *Apodemus sylvaticus*	Feeding rate	Strong initial response but rapid habituation	(Gurney *et al*., 1996)
	Meat meal MGK Big Game Repellent Feather meal Hinder Hot Sauce	White-tailed deer *Odocoileus virginianus*	Feeding rate	Yes – Strong response	(Harris *et al*., 1983)
	Thiram in the form of: Chew-not Chaperone Gustafson 42-S Spotrete-F	White-tailed deer *Odocoileus virginianus*	Feeding rate	Yes	(Harris *et al*., 1983)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Moth balls Creosote Human hair Magic circle Blood meal	White-tailed deer *Odocoileus virginianus*	Feeding rate	No or very weak response	(Harris *et al.*, 1983)
	Pulegone	Coyote *Canis latrans*	Feeding rate Behaviour	Yes	(Hoover & Conover, 2000)
	MGK Big Game Repellent	Eastern cotton-tail rabbits *Sylciagus floridanus*	Feeding rate Densities	Yes	(Mason *et al.*, 1999)
	Selinium dioxide	Swamp wallabies *Wallabia bicolor*	Feeding rate	Yes	(Montague, 1994)
	Anipel (bitrex)	Swamp wallabies *Wallabia bicolor*	Feeding rate	No	(Montague, 1994)
	Egg & paint Synthetic fermented egg Chicken manure Pulp mill liquor D-ter MGK Big Game Repellent Quazzia chips & paint Phytolacca (inkweed leaves) Bitrex Capsaicin	Swamp wallaby *Wallabia bicolor*	Browsing damage	no	(Montague *et al.*, 1990)
	Chilli paste	Swamp wallaby *Wallabia bicolor*	Browsing damage	yes	(Montague *et al.*, 1990)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Treepel	Brustail possum *Trichosurus vulpecula* Rabbit *Oryctolagus cuniculus*	Feeding rate	Yes, but response declined over 56 days	(Morgan & Woolhouse, 1995)
	Thiram Capsicum	House Mice *Mus musculus* Deer mice *Peromyscus maniculatus*	Feeding rate Behaviour	Yes	(Nolte & Barnett, 2000)
	Digitalis purpurea extract	Mountain beaver *Aplodontia rufa*	Feeding rate Behaviour	Yes	(Nolte *et al*., 1995)
	Magic circle	Beaver C*astor canadensis*	Behaviour (Dam building)	No	(Owen *et al*., 1984)
	MGK Big Game Repellent	White-tailed deer *Odocoileus virginianus*	Feeding rate	Yes	(Palmer *et al*., 1983)
	Hinder	White-tailed deer *Odocoileus virginianus*	Feeding rate	Weak response	(Palmer *et al*., 1983)
	Hot Sauce Spotrete-F Feather meal Meat meal	White-tailed deer *Odocoileus virginianus*	Feeding rate	No	(Palmer *et al*., 1983)
	Eutrofit (animal blood) Tree guard (bitrex)	Deer *Dama dama*	Feeding rate	Yes	(Santilli *et al*., 2004)
	Hot Sauce	Deer *Dama dama*	Feeding rate	No	(Santilli *et al*., 2004)
	Capsicum oleoresin	Pocket Gopher *Thomomys talpoides*	Behaviour	Yes	(Sterner *et al*., 1999)
	Quebracho Thiram	Meadow voles *Microtus pennslvanicus*	Feeding rate	Yes	(Swihart, 1990)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Methiocarb	Meadow voles *Microtus pennslvanicus*	Feeding rate	Weak response	(Swihart, 1990)
	MGK Big Game Repellent	White-tailed deer *Odocoileus virginianus*	Feeding rate	Yes	(Swihart & Conover, 1990)
	Ivory Soap	White-tailed deer *Odocoileus virginianus*	Feeding rate	Weak	(Swihart & Conover, 1990)
	Ropel	White-tailed deer *Odocoileus virginianus*	Feeding rate	no	(Swihart & Conover, 1990)
	MGK Big Game Repellent Hot sauce	Blacktailed deer *Odocoileus hemionus*	Feeding rate	Yes	(Wagner & Nolte, 2000)
	Hot sauce	Blacktailed deer *Odocoileus hemionus*	Feeding rate	Yes, but habituation occurred rapidly	(Wagner & Nolte, 2000)
	Hot Sauce	Porcupine *Erethizon dorsatum*	Feeding rate	Yes	(Wagner & Nolte, 2000)
	Hot Sauce	Pocket Gophers *Thomomys mazama*	Feeding rate	Weak response	(Wagner & Nolte, 2000)
	Hot Sauce	Mountain Beaver *Aplodontia rufa* Beaver *Castor canadensis*	Feeding rate	No	(Wagner & Nolte, 2000)
	Siberian pine needle	Deer mice *Peromyscus maniculatus* Prairie voles *Microtus ochrogaster*	Feeding rate	Yes	(Wager-Page *et al.*, 1997)
	Envirospray Ultrawax	Grey-headed flying fox *Pteropus poliocephalus*	Densities	No	(Van Der Ree & Nelson, 2002)

Origin of repellent	Odour source	Target species	Tested Effects	Repellence detected	Citation
	Tabasco sauce® Hot English mustard Indonesian fish sauce Bitrex Garlic spray D-Ter®	Brush-tail possum *Trichosurus vulpecula*	Feeding rate	No	(Cooney, 1998)
	D-Ter® Stay Off® Blood and Bone Garlic Quassia chips	Brush-tail possum *Trichosurus vulpecula*	Behaviour	No	(Cooney, 1998)
	White King® Keep Off® Camphor Naphthalene Scat®	Brush-tail possum *Trichosurus vulpecula*	Behaviour	Yes	(Cooney, 1998)
	Geranium Oil Deer Away D-pulegone	Woodchuck *Marmota monax*	Feeding and behaviour	Yes	(Bean *et al.*, 1995)
	Cinnamon leaf oil Pennyroyal oil Siberian pineneedle oil	Woodchuck *Marmota monax*	Feeding and behaviour	No	(Bean *et al.*, 1995)

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Appendix D

Additional analyses
for
Chapter 2.
Pilot screening trials with
Macropus rufogriseus banksianus

Analysis of data for individual trays
Pilot screening trial with *Macropus rufogriseus banksianus* (Chapter 2)
Consumption and approach variables

Tray A

A one-way Analysis of Variance (ANOVA) on the mass of food consumed from Tray A (Figure 1) revealed a significant difference between treatments (F[7,23]=16.06, p<0.0005). A large effect size was also apparent (eta squared =0.83). A series of *a priori* contrasts were performed (Table 1). A two-tailed contrast confirmed that no significant differences occurred between the pretrial (M=3312 g) and the procedural control (M=3275 g), water (M=3125 g) and paraffin (M=3000 g) groups. One-tailed contrasts revealed that significantly less food was consumed from the Plant Plus (M=1438 g) and Egg treatments (M=1283 g) when compared to the base group of water. No difference in the mass of food consumed at Tray A was detected between the SCAT treatment (M=3375 g) and the water group. A result approaching significance was recorded in food consumption at Tray A between IPMS (M=2375 g) and the base solvent paraffin (Table 1).

Table 1 Mass of food consumed from Tray A - *a priori* contrasts. Each row represents a contrast. Contrast 1 is two-tailed, contrasts 2-5 are one-tailed. Alpha = 0.01. Significant results are highlighted.

Contrasts: Mass of food consumed from Tray A

	Contrast Coefficients								Result			
	Pretrial	**Procedural Control**	**Water**	**Paraffin**	**SCAT**	**Egg**	**Plant Plus**	**IPMS**	**F**	**df 1**	**df 2**	**P**
1	-3	1	1	1	0	0	0	0	0.58	1	23	>0.45
2	0	0	1	0	0	0	-1	0	34.52	1	23	<0.0005
3	0	0	1	0	0	-1	0	0	35.24	1	23	<0.0005
4	0	0	1	0	-1	0	0	0	0.76	1	23	>0.19
5	0	0	0	1	0	0	0	-1	4.73	1	23	0.02

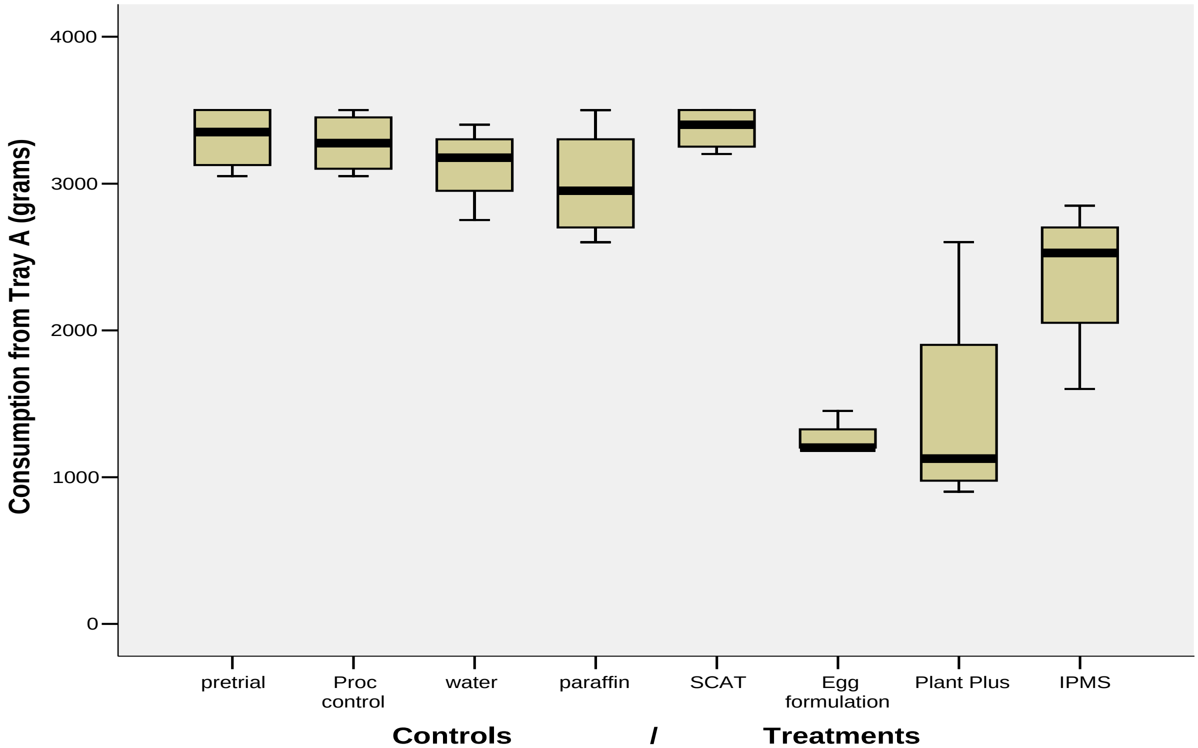

Figure 1 Boxplot of amount of food consumed from Tray A. Data is grouped by treatment. A one-way ANOVA detected a significant difference between treatments ($F[7,23]=16.06$, $p<0.0005$, eta squared $=0.83$).

A Brown-Forsythe corrected ANOVA (heterogeneity of variance was mild: Levene's statistic $[7,23]=3.3$, $p>0.01$) revealed that treatment had a significant effect on the number of approaches by *M. rufogriseus banksianus* to Feed Tray A ($F`[7,9.18]=30.53$, $p<0.0005$; Figure 2). The effect size calculated by eta squared was also large (0.90). Each row of Table 2 represents an *a priori* contrast. A two-tailed contrast confirmed that no significant differences occurred between the pretrial (M=1230) and procedural control (M=1280), water (M=1260) and paraffin (M=1243) groups. One tailed contrasts revealed that significantly fewer approaches were made by *M. rufogriseus banksianus* to Feed Tray A for each of the Plant Plus (M=38) and Egg treatments (M=290) when compared to the water group. No difference in the number of approaches to Tray A was detected between the SCAT treatment (M=1371) and the water group. Additionally, no significant difference was detected in the number of *M. rufogriseus banksianus* approaches between the IPMS (M=963) and paraffin groups (Table 2).

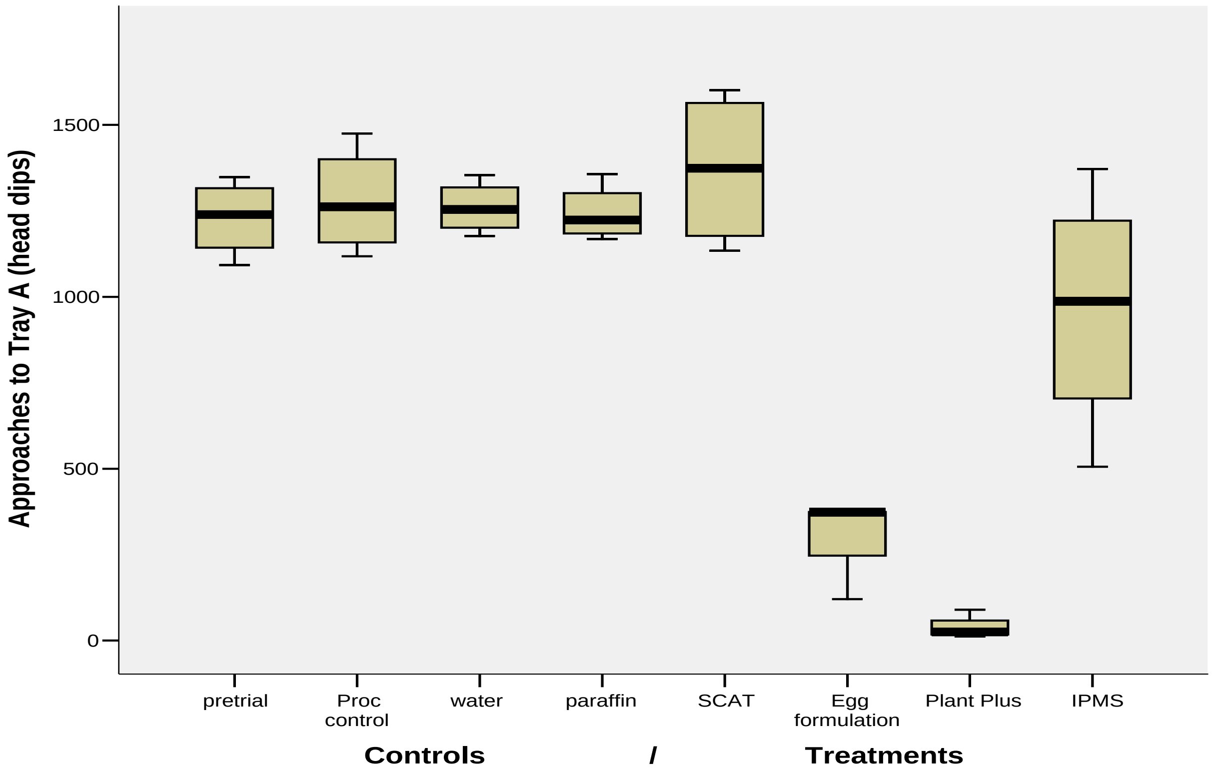

Figure 2 Boxplot of *M. rufogriseus banksianus* approaches (head dips) to Tray A. Data is grouped by treatment. A one-way ANOVA with Brown-Forsythe correction for heterogeneity of variance detected a significant difference between treatments (F`[7,9.18]=30.53, p<0.0005, eta squared =0.90).

Table2 Approaches to Tray A - *a priori* contrasts. Each row represents a contrast. Contrast 1 is two-tailed, contrasts 2-5 are one-tailed. Alpha = 0.01. Significant results are highlighted.

Contrasts: Approaches to Feed Tray A

	Contrast Coefficients								Result			
	Pretrial	**Procedural Control**	**Water**	**Paraffin**	**SCAT**	**Egg**	**Plant Plus**	**IPMS**	**F**	**df 1**	**df 2**	**P**
1	-3	1	1	1	0	0	0	0	0.23	1	5.0	>0.64
2	0	0	1	0	0	0	-1	0	845.53	1	4.2	<0.0005
3	0	0	1	0	0	-1	0	0	109.39	1	2.8	<0.002
4	0	0	1	0	-1	0	0	0	0.85	1	3.7	>0.20
5	0	0	0	1	0	0	0	-1	2.29	1	3.3	>0.14

Tray B

A significant difference in the mass of food consumed from Tray B (Figure 3) was detected between treatments with a one-way ANOVA ($F[7,23]=9.71$, $p<0.0005$). A large effect size was also apparent (eta squared=0.75). Table 3 contains a series of *a priori* contrasts that were performed. A two-tailed contrast confirmed that no significant differences occurred between the pretrial (M=2063 g) and the procedural control (M=1950 g), water (M=1812 g) and paraffin (M=1875 g) groups. One-tailed contrasts revealed that significantly more food was consumed from Tray B for both of the Plant Plus (M=3313 g) and Egg treatments (M=3267 g) when compared to the water group. No difference in the mass of food consumed at Tray B was detected between the SCAT treatment (M=1950 g) and the water group. No significant difference in feeding at Tray B was detected between the IPMS (M=2225 g) and paraffin groups (Table 3).

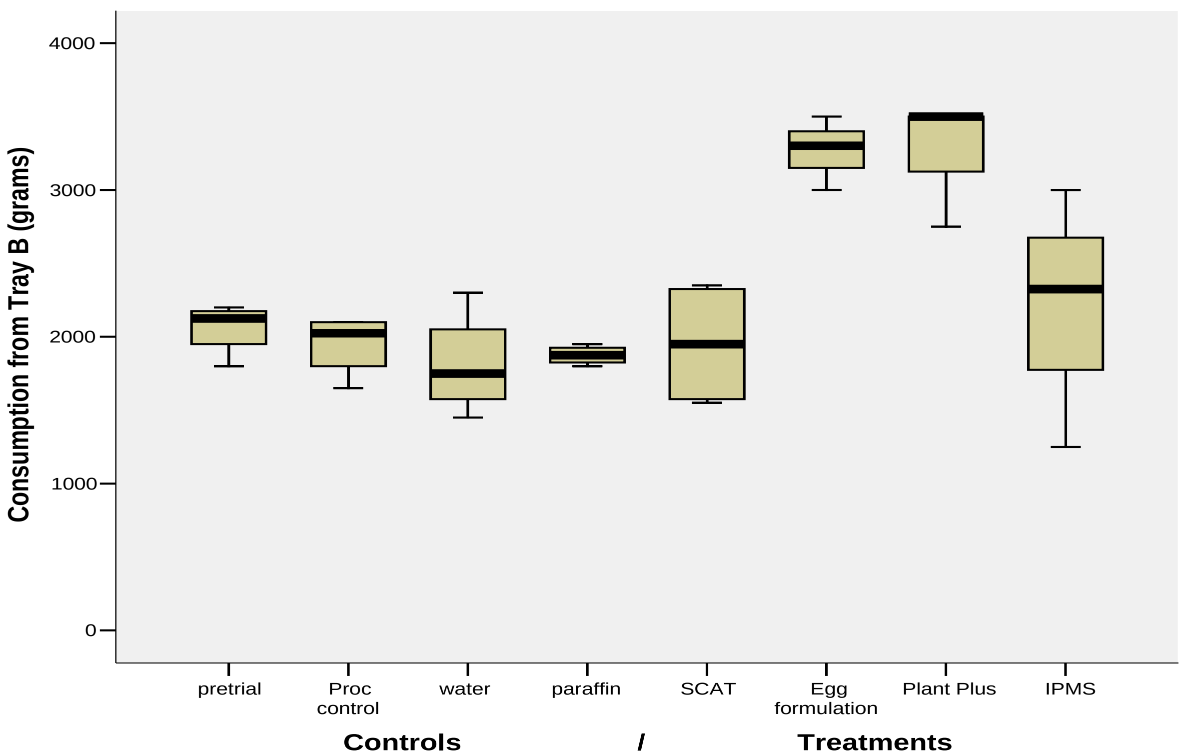

Figure 3 Boxplot of amount of food consumed from Tray B. Data is grouped by treatment. A one-way ANOVA detected a significant difference between treatments ($F[7,23]=9.71$, $p<0.0005$, eta squared =0.75).

Table 3 Mass of food consumed from Tray B - *a priori* contrasts test specific hypotheses: Each row represents a contrast. Contrast 1 is two-tailed, contrasts 2-5 are one-tailed. Alpha = 0.01. Significant results are highlighted.

Contrasts: Mass of food consumed from Tray B

	Contrast Coefficients								Result			
	Pretrial	**Procedural Control**	**Water**	**Paraffin**	**SCAT**	**Egg**	**Plant Plus**	**IPM S**	**F**	**df 1**	**df 2**	**P**
1	-3	1	1	1	0	0	0	0	0.70	1	23	>0.41
2	0	0	1	0	0	0	-1	0	31.25	1	23	<0.0005
3	0	0	1	0	0	-1	0	0	25.17	1	23	<0.0005
4	0	0	1	0	-1	0	0	0	0.26	1	23	>0.30
5	0	0	0	1	0	0	0	-1	1.70	1	23	>0.10

A Brown-Forsythe corrected ANOVA detected a significant difference between treatments in the number of *M. rufogriseus banksianus* approaches (head dips) to Feed Tray B (F'[7,7.41]=16.47, p<0.001, Levene statistic [7,23]=2.6, p>0.01; Figure 4). A large effect size was also apparent (eta squared =0.83). A series of *a priori* contrasts were performed (Table 4). A two-tailed contrast revealed a result approaching significance occurred between the pretrial (M=475) and procedural control (M=719), water (M=538) and paraffin (M=675) groups. Further analysis revealed the pretrial and the procedural control groups were most dissimilar of the control groups and the water and paraffin groups were intermediary. All control groups were pooled to ensure that variance in the control groups was maintained, reducing the chance of a spurious treatment effect. A significantly larger number of approaches by *M. rufogriseus banksianus* were made during the Plant Plus (M=1624) and Egg treatments (M=1625) when compared to all control groups combined. No difference was detected between either the SCAT (M=545) and control groups or the IPMS (M=813) and control groups (Table 4).

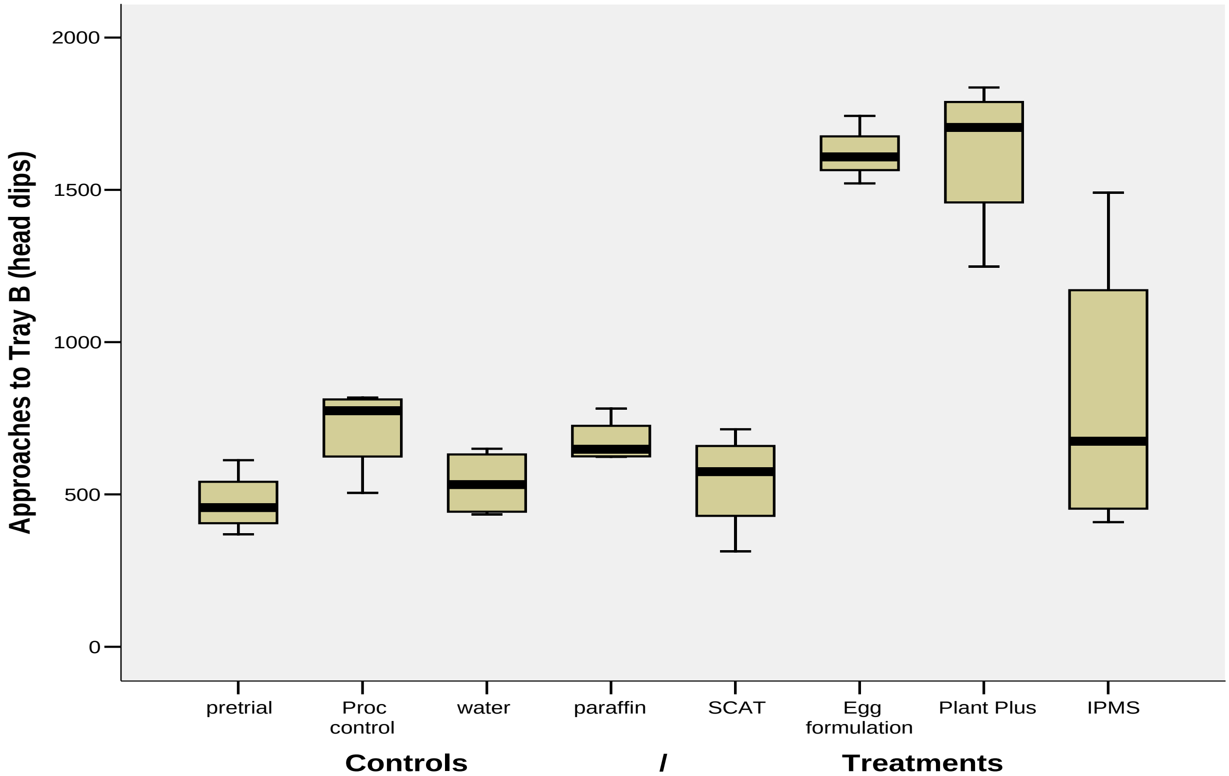

Figure 4 Boxplot of *M. rufogriseus banksianus* approaches (head dips) to Tray B. Data is grouped by treatment. A one-way ANOVA with Brown-Forsythe correction for heterogeneity of variance detected a significant difference between treatments (F`[7,7.41]=16.47, p<0.001, eta squared =0.83).

Table 4 Approaches to Feed Tray B - *a priori* contrasts were run to test specific hypotheses: Each row represents a contrast. Contrast 1 is two-tailed, contrasts 2-5 are one-tailed. Alpha = 0.01. Significant results are highlighted.

Contrasts: Approaches to Feed Tray B

	Contrast Coefficients								Result			
	Pretrial	**Procedural Control**	**Water**	**Paraffin**	**SCAT**	**Egg**	**Plant Plus**	**IPMS**	**F**	**df 1**	**df 2**	**P**
1	-3	1	1	1	0	0	0	0	7.85	1	5.6	>0.03
2	-1	-1	-1	-1	0	0	4	0	59.46	1	3.3	<0.0015
3	-1	-1	-1	-1	0	4	0	0	213.63	1	2.8	<0.0005
4	-1	-1	-1	-1	4	0	0	0	0.41	1	3.7	>0.28
5	-1	-1	-1	-1	0	0	0	4	0.73	1	3.1	>0.22

Appendix E

Summary data for Chapter 5. Longevity of an odorous repellent with *Macropus rufogriseus banksianus*

Summary data for Consumption and Approach variables
Chapter 5 Longevity of an odorous repellent with *Macropus rufogriseus banksianus*.

Summary of data relating to consumption at feed stations: Longevity trial

Treatment	*n*	*Treated tray*	*Untreated tray*	*Total* *(treated+untreated)*	*Difference* *(treated-untreated)*	*Preference* *treated/total*
		Mean (standard error)				
1-week	3	1733 (83)	2267 (60)	4000 (132)	-533 (60)	0.43 (0.01)
10-weeks	3	1783 (187)	2283 (73)	4067 (240)	-500 (153)	0.43 (0.02)
22-weeks	3	2033 (88)	2350 (161)	4383 (220)	-317 (136)	0.46 (0.02)
32-weeks	3	2067 (289)	2250 (76)	4316 (233)	-183 (353)	0.47 (0.04)

Summary of data relating to approaches to feed stations: Longevity trial

Treatment	*n*	*Treated tray*	*Untreated tray*	*Total* *(treated+untreated)*	*Difference* *(treated-untreated)*	*Preference* *treated/total*
		Mean (standard error)				
1-week	3	450 (19)	499 (34)	949 (52)	-50 (22)	0.47 (0.01)
10-weeks	3	625 (41)	908 (54)	1533 (13)	-283 (95)	0.41 (0.03)
22-weeks	3	486 (10)	537 (31)	1023 (35)	-51 (30)	0.48 (0.01)
32-weeks	3	576 (52)	575 (18)	1151 (35)	0 (69)	0.50 (0.03)